Editorial

New Frontiers in Cardiology

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Every discipline, whether in science, the humanities or the arts, reaches a point in its evolution where progress becomes slow and rewarding only to those who are content to preserve the status quo. It is at this point that imagination and enthusiasm, searching for new frontiers, find new approaches which widen the artistic or scientific horizon. In science, these approaches are often made possible through technical advances.

Cardiology has, until about fifteen years ago, consisted primarily of clinical observations supplemented by electrocardiography. Since 1944, the advent of cardiac catheterization and angiocardiography has lead to considerable advances. The diagnostic application of these techniques together with the development of cardiac surgery have made cardiology one of the most rapidly growing fields in internal medicine. But the impetus of this new technique with its practical gains is beginning to slow down and new approaches, new viewpoints are needed. There are signs that cardiology is again on the road, moving at an accelerated pace. It is the purpose of this editorial to outline some of these new frontiers along with the progress being made.

In dealing first with technical advances, the use of dye-dilution methods combined with right and left heart catheterization has aided in the localization of defects and shunts.[1)a,b] Other new methods for the diagnosis and localization of shunts such as the nitrous oxide method,[2)] the use of radioactive krypton,[3)] the hydrogen electrode[4)] and the use of external precordial counting systems[5)] are influencing the accuracy of preoperative diagnosis. Selective angiocardiography, particularly cinecardioangiography promises to be not only of diagnostic value but also of particular physiologic importance. In cinecardioangiography the passage of contrast material is recorded by means of x-ray motion picture photography. The success of this method has been made possible by the development of the image amplifier, which increases the brightness of the fluoroscopic screen from 200 to over 1000 times. The widest application

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of this technique is likely to be in the field of coronary artery disease.[5] The method makes possible direct and momentary visualization of the coronary arteries and permits the recognition of coronary pathology; it will be helpful in the understanding of coronary hemodynamics.

If the future of cardiology were to depend entirely on technical advances, it would cease to be a science. It is fortunate, therefore, that new frontiers are opening up in the field of cardiology as related to biochemistry and to biophysics. Within recent years the properties of the contractile elements of heart muscle have been investigated with the ultimate goal of defining possible defects in either the structure or biophysical properties which may be responsible for disturbed cardiac function in congestive heart failure. By studying relative viscosity, ATP-ase activity, light scattering and sedimentation constants of contractile proteins, important information has been obtained.[6],[7] The structure of cardiac muscle cell has been explored and found to be entirely comparable to that of skeletal muscle.[8] The sarcoplasmic reticulum, a system of anastomosing membrane-limited channels which traverse the interior of the myocardial cells is particularly well developed in the myocardium. Its function appears to be connected with transport of metabolites and with the conduction of electrical impulses into the interior of the cell.[9] It is particularly interesting that the sarcoplasmic reticulum may be the site of synthesis or storage of the relaxing factor, which has recently assumed such great importance for cardiology. For example, Lee and his co-workers have pointed to the importance of the relaxing factor in the mechanism of the action of digitalis.[10] Based on electromicroscopy, it has been suggested that contraction of skeletal and heart muscle is accompanied by the movement of thin filaments relative to thick filaments in muscle contraction. The thin filaments were proposed as actin, the thick filaments as myosin.[11] This "sliding filament hypothesis" is interesting but needs further investigation before it can be accepted.

Work on cardiac metabolism has a more than casual relationship to the problems faced by the clinical cardiologist. Using coronary sinus catheterization, workers in this country and in Japan have shown the myocardial utilization of glucose, pyruvate, lactate, ketone bodies, amino acids and fatty acids.[12],[13],[14] In the postabsorptive state, about three-fifths of the energy expenditure of the heart is sustained by fatty acids, primarily esterified fatty acids.[12] Using gas chromatography, significant differences were found between the free fatty acid composition of arterial and coronary venous blood.[12] Apparently, although the human and dog heart extracts all individual free fatty acids, there were significant differences in the rate of their myocardial uptake. This was indicated

by the relative larger percentage of myocardial extraction of oleic acid. Studies of the metabolism of the anoxic heart have elucidated the biochemical chain of events which take place in such clinical conditions as ventricular tachycardia and fibrillation.[12] These conditions have been shown to lead to a transitory increase in the ratio phosphorylase a to total phosphorylase.[12] In addition, cardiac glycogen diminished and there was a slight rise in glucose-6-phosphate, a marked elevation in lactic acid. When ventricular and atrial fibrillation and ventricular tachycardia were produced in hearts in which the coronary circulation was maintained, no alterations in carbohydrate intermediates and phosphorylase a activity were observed.[12] Apparently, increased heart rate in the absence of anoxia failed to evoke the classic pattern resulting from increased rate of stimulation described as typical for skeletal muscle. Similar studies have shown the importance of myocardial anoxia in the production of hemorrhagic shock, coronary embolization and myocardial infarction. Studies on the metabolism of the failing heart have demonstrated that there are two types of heart failure, non-metabolic and metabolic failure. In the former, disturbances in energy production are absent while in the latter, such as in heart failure from anemia, anoxia, hyperthyroidism or thiamine deficiency, definite metabolic disturbances can be found.[12]

Of interest have been the studies on the metabolism of the transplanted heart as carried out by Chiba, Chrysohou, Gudbjarnason and Wolf.[15] These workers have demonstrated that the metabolic changes occurring in the transplanted heart are the result of permeability of the cell of the transplant resulting in loss of enzymes and coenzymes from the heart muscle cell. Histologically, these hearts show lesions resembling in many respects those found in acute rheumatic fever with Aschoff-like nodules and granulomatous myocarditis.[15]

This editorial has, it is hoped, shown that cardiology is not only a clinical discipline, but a live science, the frontiers of which are constantly expanding.

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Treatment of Arteriosclerosis
Vitamins and Sulfuric Esters of Polysaccharides

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The investigation on Vitamin B_6 and lipemia clearing factor relating to the etiology and treatment of arteriosclerosis is described in this paper.

Pyridoxal phosphate seems to be a co-enzyme of lipemia clearing factor. Arteriosclerosis in the Vitamin B_6 deficient monkey was very similar to that in the human. The prophylaxis and treatment with pyridoxal phosphate for experimental atherosclerosis in rabbits was possible. The dextran sulfate which is low in the anticoagulant activity and high in the lipemia clearing activity was synthesized at our laboratory and the prophylaxis with this dextran sulfate for experimental atherosclerosis in rabbits was reported. The clinical applications of pyridoxal phosphate and this dextran sulfate were also demonstrated. In addition, some considerations on the oral administration of dextran sulfate were made.

THE serum cholesterol level did not alter in the Vitamin B_6 deficient dog, rhesus monkey and cebus monkey,[1),2)] but it was found by Dann[3)] that serum cholesterol increased in the Vitamin B_6 deficient chicken. Failey[4)] reported that serum cholesterol decreased by the addition of Vitamin B_6 on that occasion. Yamamoto[5)] in my laboratory reported that Vitamin B_6 deficiency accelerated the atherosclerotic changes in the albino rats fed the atherogenic diet. From the following findings that Vitamin B_6 is required for the efficient conversion of linoleic acid to arachidonic acid, that Vitamin B_6 and unsaturated fatty acids act efficiently ***in vivo***[6)] and that iodine number decreased in the Vitamin B_6 deficiency, it is conceivable that there may be some intimate relationship between Vitamin B_6 and lipid metabolism. Furthermore, the action of Vitamin B_6 as a co-enzyme of decarboxylase in the synthetic process of CoA,[8)] the elevation of serum cholesterol in the liver of Vitamin B_6 deficient rats, the increase in acid soluble inorganic phosphorus and the decrease in acid

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soluble organic phosphorus in the liver of Vitamin B_6 deficient rats, the lowering of oxidative phosphorylation, the enhancement of ATPase activity and the definite decrease in ⊿7P in the liver of Vitamin B_6 deficient rats[7] were investigated. These findings indicate that there are the inhibition in the synthesis of organic phosphorus from inorganic phosphorus, the acceleration in the degradation of organic phosphorus and particularly the considerable disturbances in the high energic phosphorus metabolism. We have reported at the 33rd General Meeting of Japan Endocrine Society[9] that noradrenalin combined with pyridoxal phosphate and interfered the Vitamin B_6 enzyme *in vitro* and that DOPA and DOPA-amine interfered the Vitamin B_6 enzyme in similar to noradrenalin.

As mentioned above, I have insisted that Vitamin B_6 has a considerable relationship with the etiology of arteriosclerosis. In this report, the prophylaxis and treatment of arteriosclerosis with Vitamin B_6 and sulfuric esters of dextran are discussed in relation to lipemia clearing factor.

Lipemia Clearing Factor and Vitamin B_6

I have studied on the relationship between lipemia clearing factor (C. F.) and Vitamin B_6 during the last few years. I ascertained that pyridoxal phosphate (PAL-PO_4) has the character likely a co-enzyme of C. F. by the ultraviolet absorption of purified C. F. and by the experiment in which PAL-PO_4 was added *in vitro*.[10] Engelberg[11] stated on the significance of heparin in relation to C. F. that heparin was a co-enzyme of it. On the other hand, Robinson et al.[12] proposed that heparin influenced on only the stability of C. F. enzyme and that C. F. was released from the capillary surface into the blood stream by the injection of heparin. Spitzer et al.[13] reported that C. F. was contained in the albumin fraction of plasma and also in our experiment[10] the purified C. F. enzyme was noticed in the albumin fraction. However, Eiber et al.[14] who injected S^{35} labeled heparin to the dog and pursued the distribution of S^{35} in the plasma protein, reported only the trace of it in the albumin fraction. The reason why the significance of heparin as a co-enzyme of C. F. enzyme is doubtful is that the C. F. enzyme was contained in the albumin fraction and S^{35} labeled heparin was not noticed in the albumin fraction.

Arteriosclerosis in the Vitamin B_6 Deficient Monkey

Rinehart et al.[15] succeeded in producing arteriosclerosis of rhesus monkey without any additional fat in 1948. They proved that this arteriosclerotic change was due to neither the inanition nor the deficiency of 2 or 3 vitamins or any other nutrients, since the initial changes of arteriosclerosis were found in the 5th month of deficiency. I pursued the occurring of arteriosclerosis in the Vitamin B_6 deficient Japanese

monkey, as the Vitamin B_6 deficiency was ascertained by the secretion of xanthurenic acid in the urine and the Vitamin B_6 level in the blood. Plate 1 shows the iliac artery in the 10th month of deficiency, Plate 2 shows small artery in the heart muscle of the same animal and Plate 3 shows small artery in the heart muscle of the 12th month. In Plate 3, the artery was occluded entirely. In the abdominal aorta of the same animal shown in Plate 4, the formation of plaque was remarkable. Plate 5 shows the arteriosclerosis of small arteries in the pelvis of the same animal. Marked arteriosclerotic pictures were also seen in the arteries of pancreas, kidney and testes. The changes of ST and T in the E. C. G. in these monkeys were noticed. As long as I judge from my results up to present, these monkeys did not suffer from diabetes mellitus. In other words, I think the arteriosclerosis in the Vitamin B_6 deficiency is not produced through diabetes.

Prophylaxis and Treatment of Experimental Atherosclerosis with Vitamin B_6

Although I already mentioned the experiments made by Faily and Dann in chicken, Martens et al.[16] reported that pyridoxine had no influences on serum cholesterol and the development of atherosclerosis in the rabbits fed cholesterol. I noticed some preventive effects of PAL-PO_4 and pyridoxamine phosphate (PAM-PO_4) on the atherosclerosis of the rabbits fed lanolin 5 mg. a day, while pyridoxine had no effects. We already informed these findings at the 24th General Meeting of Japanese Circulation Society.[17] This time my co-workers and I investigated the effects of PAL-PO_4 and pyridoxine on the treatment of atherosclerosis in the rabbits fed lanolin for 16 weeks. Fig. 1 shows total cholesterol in the serum. If the feeding of lanolin was discontinued, the high cholesterol level decreased rapidly even in the control group, but it decreased more markedly in the PAL-PO_4 group. However, in the pyridoxine group there were no significant differences between the treated group and the control group. The same results were also obtained in free cholesterol and the c/p ratio. Plate 6 shows the fat stain of aorta in the control rabbit and Plate 7 shows that the changes of aorta in the treated animal were alleviated with PAL-PO_4. Furthermore, we studied on the effects of PAL-PO_4 and pyridoxine on the rabbits fed lanolin for 12 weeks succeeding to 16 weeks. As shown in Fig. 2, the control group maintained the high cholesterol level continuously but in the PAL-PO_4 group the cholesterol level declined gradually. There were no significant effects in the pyridoxine group. The same results were also obtained in free cholesterol and the c/p ratio. Plate 8 shows the elastic fiber staining of aorta in the control rabbit, and Plate 9 shows the aorta in the treated animal with PAL-PO_4. As mentioned above, the effects of PAL-PO_4 on

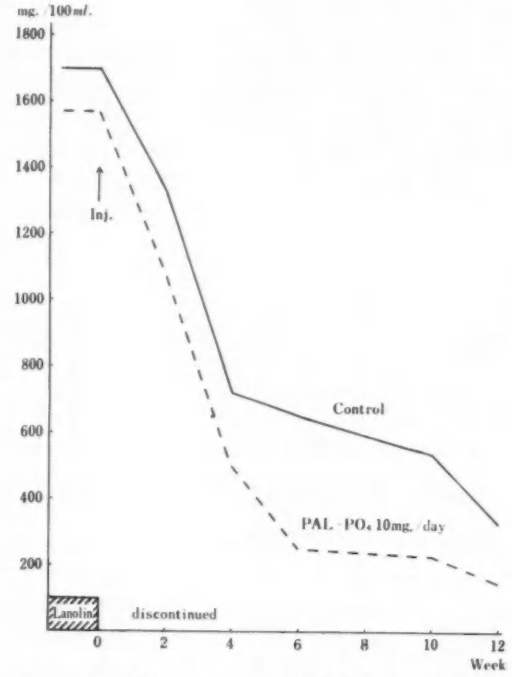

Fig. 1. Effect of PAL-PO_4 on serum total cholesterol of rabbits fed lanolin

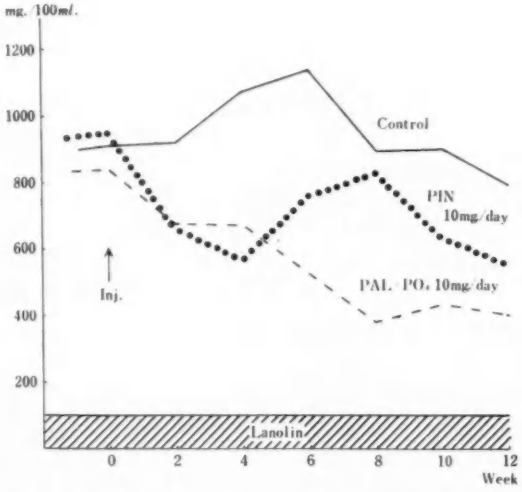

Fig. 2. Effect of PAL-PO_4 and PIN on serum total cholesterol of rabbits fed lanolin.

the treatment of experimental atherosclerosis were apparently obtained, although these effects were less than those of prophylaxis. However, there were no noticeable effects in the pyridoxine group. These findings indicate the importance of Vitamin B_6 for the development of atherosclerosis and the inhibition in the activation of Vitamin B_6 associated with the disturbances of phosphorylation.

The Treatment of Arteriosclerosis with Vitamin B_6 in Man

From the effects of PAL-PO_4 on the experimental atherosclerosis, PAL-PO_4 5 mg. a day for 20 days followed by pyridoxine 20 mg. a day for 15 days were given to the patient who were supposed to be arteriosclerotic and the effects in these 2 periods were compared. The marked decrease in serum total cholesterol, the increase in C. F. and the improvement of serum protein pattern and electrocardiographic findings resulted from the administration of PAL-PO_4, but there were no significant effects by pyridoxine. These findings were reported at the first General Meeting of Japan Aging Association.[18] Although PAL-PO_4 had favorable effects on the treatment of arteriosclerosis for a short duration in man, considering the general property of vitamin, whether there are good results on the treatment for a long duration or not is to be reevaluated. I evaluated in the cases administered PAL-PO_4 every day or every other day for 4 to 12 months. As shown in Fig. 3, the serum cholesterol

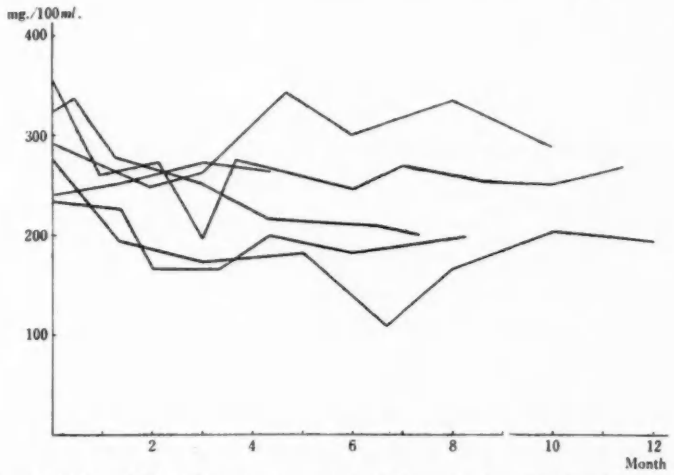

Fig. 3. Effect of PAL-PO_4 on serum total cholesterol of arteriosclerotic patients.

decreased in the beginning, but there were no tendency to decrease after then. These findings were also seen in the phospholipids fraction and the c/p

ratio. The serum protein pattern was also improved but it was attributed to the increase in albumin and not to globulin. As shown in Fig. 4,

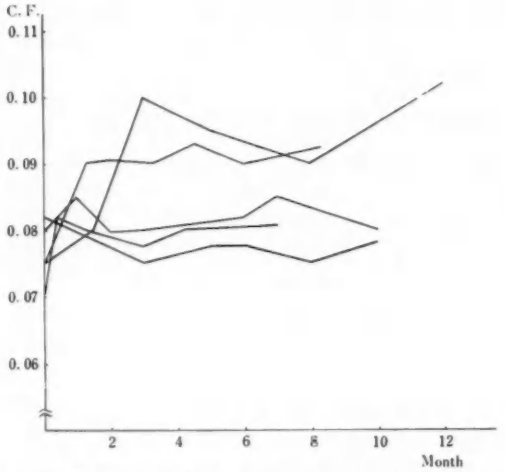

Fig. 4. Effect of PAL-PO_4 on lipemia clearing factor of arteriosclerotic patients.

the activity of plasma C. F. was accelerated in the beginning and then maintained during the PAL-PO_4 administration. In spite of the administration for a long duration, the improvement of eyeground, kidney function and E. C. G. was not marked. Thus the long term treatment of human arteriosclerosis with PAL-PO_4 was effective, but it was not conclusive as in the animal experiment of a short duration. This indicates the difficulty of treatment for arteriosclerosis. Anyhow, it seems to me that more cases should be added before any definite conclusion is drawn.

Basal Experiments on Clinical Application of Sulfuric Esters of Polysaccharides, Particularly Dextran Sulfate

From the evidence that heparin contains sulfuric group which activates the C. F. activity, it was found that sulfuric esters of many other polysacchrides have the activity to produce C. F. For example, sulfuric ester of alginic acid, dextran sulfate, sulfuric ester of thioctic acid, sulfuric ester of xylan and laminarin sulfate etc. have such activity. These sulfuric esters of polysaccharides are less expensive, because these are partially synthesized on the contrary to heparin which extracted from living body. So here is another reason that the clinical application of these is desirable. For the same purpose, we compared the producibility of C. F. by sulfuric esters of a few of polysaccharides, and found that

sulfuric esters of alginic acid, raffinose and dextran had considerable producibility of C. F. by a certain dosage. Sucrose and Sorbitol were ineffective. We reported above findings at the first General Meeting of Japan Aging Association.[18] As sulfuric ester of polysaccharides has the anticoagulant activity (ACA), what kind of relation exists between the producibility of C. F. and ACA is an interesting problem, considering the essential of C. F. producibility. I compared the C. F. producibility of dextran sulfate which had various sulfate content, molecular weight and ACA as various dextran sulfates with different molecular weight were gained easily. By Fig. 5, we can understand that the dextran sulfate,

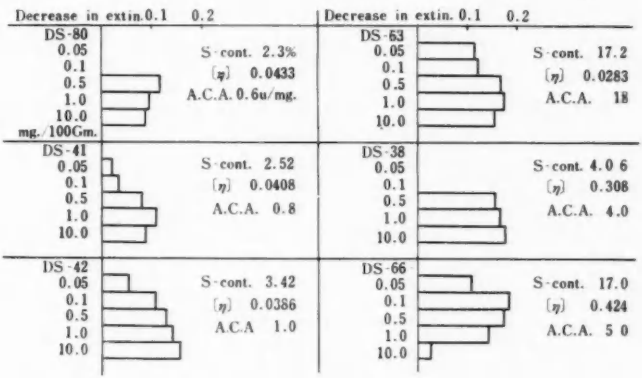

Fig. 5. Clearing activity induced by various dextran sulphates injected in varying amounts into rats.

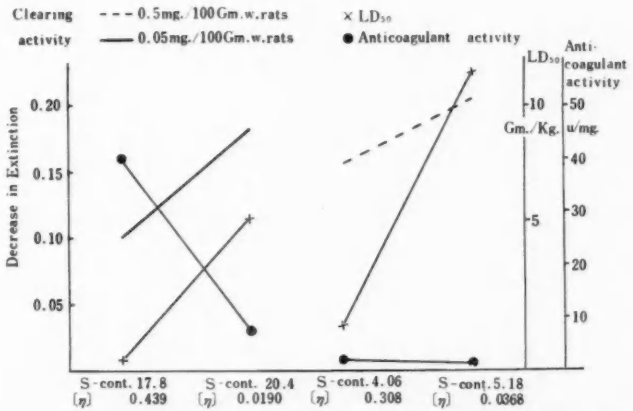

Fig. 6. Clearing activity, anticoagulant activity and LD_{50} of dextran sulphates which have various viscosity in constant S-content.

of which molecular weight is low and the sulfate content is a little, has sufficient producibility of C. F. in the proper dosage and low ACA. In other words, the dextran sulfate which is low in the ACA and has the C. F. producibility can be synthesized. Fig. 6 shows the relationship among C. F. producibility, ACA and LD_{50} in the dextran sulfate which contains the same amount of sulphur and how their relationship changed when molecular weight, that is to say, intrinsic viscosity [η] was altered to extremes. When the sulphur content was from 17 to 20%, the lower the molecular weight, the higher the LD_{50} and the lower the ACA. On the contrary, the C. F. producibility increased. This suggested that the mechanisms of C. F. producibility and ACA of dextran sulfate are not the same. In the dextran sulfate which contains 4 to 5% of sulphur, the ACA was mostly none. And the lower the molecular weight, the higher the LD_{50}. But the C. F. producibility was not noticed in these. However, if dosages were increased, the C. F. producibility was apparently noticed. If we varied the sulphur content of dextran sulfate, of which intrinsic viscosity was around 0.03 and molecular weight was low, the ACA increased rapidly in sulphur content of 14 to 17% and not increased further in sulphur content of over 17% (Fig. 7). From above evidences, the dextran

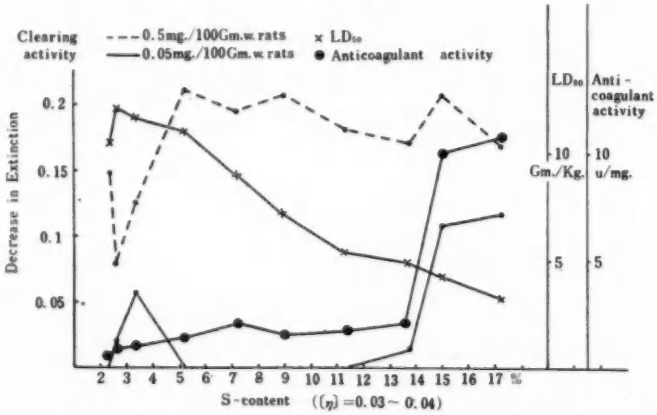

Fig. 7. Clearing activity, anticoagulant activity and LD_{50} of dextran sulphates which contain various sulphur and have similar viscosity.

sulfate (D. S.), of which the molecular weight is low, intrinsic viscosity is around 0.03 and the sulphur content is 2 to 5%, is high in the C. F., less toxic and very low in the ACA. Thus the possibility of clinical application of this D. S. was indicated. On clinical application, I admitted that D. S. is superior to heparin in various points by comparing with heparin of the same unit of ACA. I already reported these results at the

Plate 1. Iliac artery of B_6-deficient monkey (Elastica Van-Gieson staining).

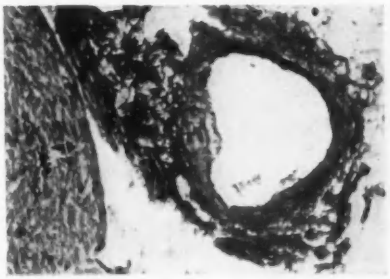

Plate 2. Small artery of heart of B_6-deficient monkey (Van-Gieson staining).

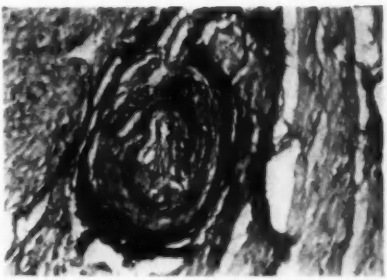

Plate 3. Small artery of heart of B_6-deficient monkey (Van-Gieson staining).

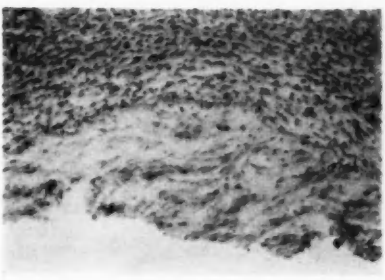

Plate 4. Abdominal aorta of B_6-deficient monkey (Sudan III staining).

Plate 5. Small artery in pelvis of B_6-deficient monkey (Elastica Van-Gieson staining).

Plate 6. Aorta of control rabbit discontinued lanolin feeding (Sudan III staining).

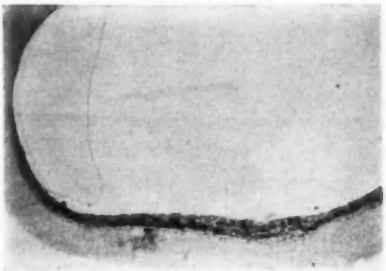

Plate 7. Aorta of rabbit discontinued lanolin feeding and treated by PAL-PO_4 (Sudan III staining).

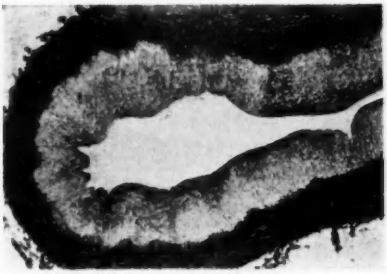

Plate 8. Aorta of control rabbit continued lanolin feeding (Van-Gieson staining).

Plate 9. Aorta of rabbit continued lanolin feeding and treated by PAL-PO_4 (Van-Gieson staining).

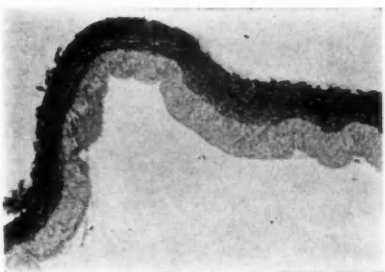

Plate 10. Aorta of control rabbit (H. E. staining).

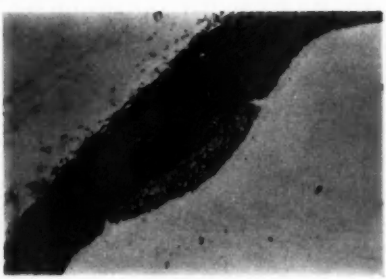

Plate 11. Aorta of rabbit treated by dextran-sulphate (Toluidineblue staining).

24 th General Meeting of Japanese Circulation Society.[17]

Prophylaxis of Experimental Arteriosclerosis with Dextran Sulfate

Constantinides et al.[19] noticed the anti-atherosclerotic activity of sulfuric ester of alginic acid and explained this phenomenon that this sulfuric ester stimulated the phagocytosis of reticulo-endothelial system. Oshima et al.[20] noticed the antiatherogenic activity of laminarin sulfate containing 18% of sulphur and also evaluated sulfuric esters of a few of polysaccharides. Now I thought it is an interesting subject whether the D. S. mentioned in the previous chapter may prevent atherosclerosis or not besides the C. F. producibility *in vivo*. I studied the prophylaxis of atherosclerosis with D. S. in the rabbits fed lanolin. As shown in Fig. 8,

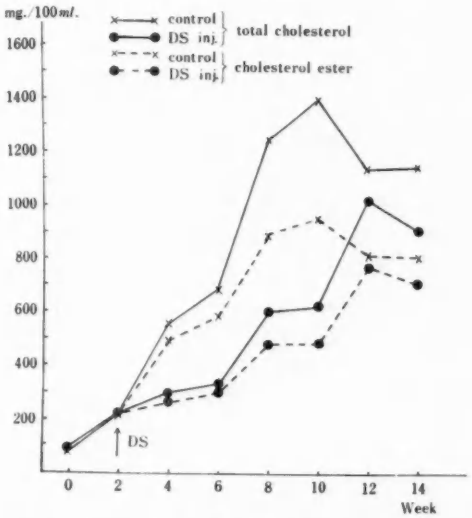

Fig. 8. Total cholesterol and cholesterol ester in lanolin-fed and lanolin-DS-treated rabbits.

total and ester cholesterol in the serum increased gradually but in the group injected D. S. the cholesterol level was considerably suppressed. Phospholipids and the c/p ratio were suppressed to a certain extent. As shown in Plate 10 and 11, the histological changes of aorta after 14 weeks were markedly mild in the group injected D. S. There was no evidence that metachromasia positive substance increased in the D. S. treated group comparing with the control group. As mentioned above, D. S. showed sufficient result in the prophylaxis of experimental atherosclerosis.

Clinical Application of Dextran Sulfate

From the results mentioned in the previous chapter, I applied the D. S., of which intrinsic viscosity was low and sulphur content was low, to arteriosclerosis in man. When 600 mg. of D. S. was injected separately 2 times a day, total and ester cholesterol decreased in most cases after about 2 weeks, but after then the cholesterol level started to increase and returned to the previous level in some of them. Cohen et al.[21] already noticed this phenomenon on D. S. which contained considerable amount of sulphur. On this occasion, if the dosage was decreased to a half and it was given every other day, the cholesterol level decreased again. The cholesterol level, which increased by the administration of every other day, decreased by the administration of everyday in some cases. In Fig. 9 showing the changes of free cholesterol with D. S. injec-

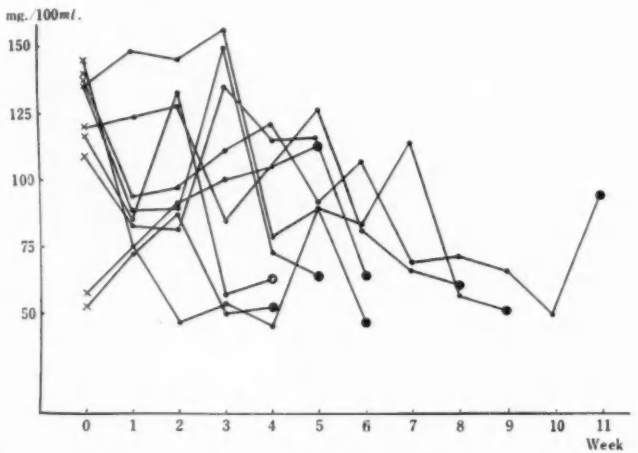

Fig. 9. Effect of intravenous administration of dextran sulphate on serum free cholesterol.

tion, the decrease of free cholesterol is more marked than that of total cholesterol. Although it was investigated in Fig. 10 whether there were some differences in the effects of D. S. on C. F. in the cases in which cholesterol did not decrease with administration of D. S. and in other cases in which cholesterol decreased, there was no decrease in C. F. in both cases. From above results, it is apparent that C. F. producibility does not decrease by the administration for at least 4 weeks. Anyhow, we should further evaluate the administration method of D. S. but it is not doubtful that it has good influence on the lipid metabolism in aged person.

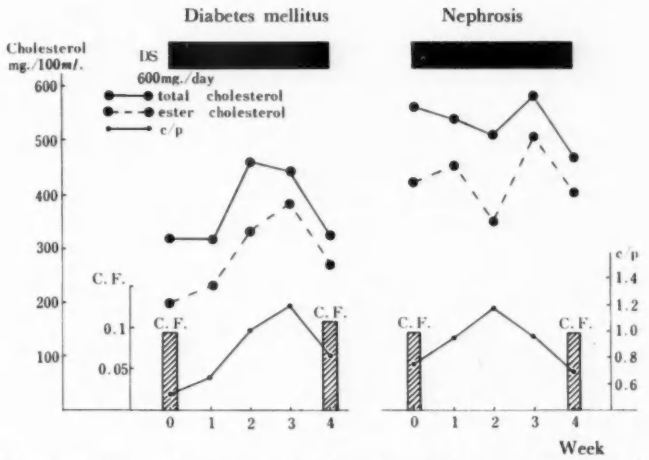

Fig. 10. Effect of intravenous administration of dextran sulphate on clearing activity and serum lipid in men.

Intestinal Absorption of D. S.

Although it has been recognized that heparin or heparin-like substances are effective by the extraoral administration, the buccal absorption which was tried recently[22] is noteworthy. Loomis[23] showed that heparin is not broken down in the intestine of dog and the absorption through the intestine was very little. They desulfated or methylated heparin partially and demonstrated that it was absorbed in the normal pH in the intestine.[24] From the evidences that D. S. is a extremely short chaining polymer compared with heparin and has no carboxyl group, I supposed that D. S. is absorbed more easily than heparin in the intestine.

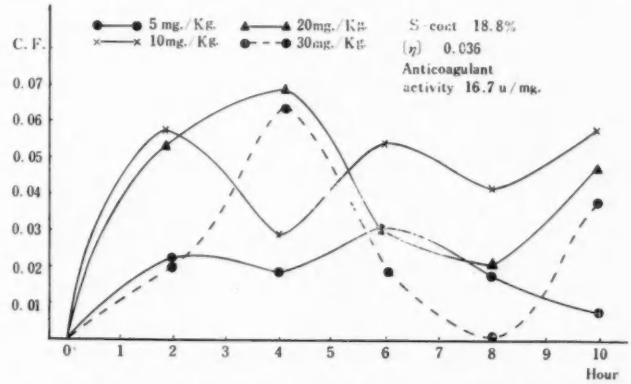

Fig. 11. Clearing activity induced by oral administration of dextran sulphate in dogs.

Fig. 11 shows the changes in C. F. in the plasma observed when the D. S., of which the sulphur content is 18% and the molecular weight is low, was given in various dosages. In any dosage, the C. F. level increased apparently, that is to say, it showed that D. S. was absorbed. However, 5 mg. per Kg. of body weight is insufficient and over 10 to 20 mg. per Kg. body weight is proper. In Fig. 12 it was shown that the D. S., of

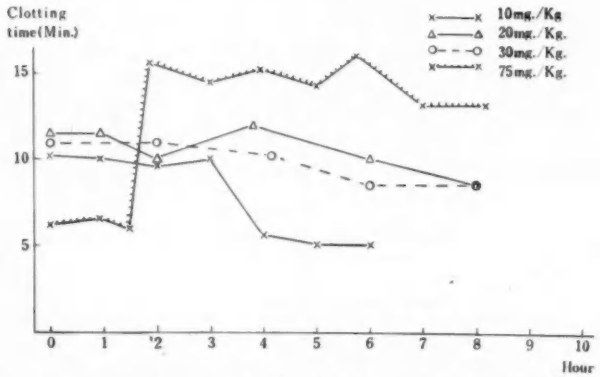

Fig. 12. Anticoagulant activity induced by oral administration of dextran sulphate in dogs.

which the sulphur content is 18% and the ACA is relatively high, had the C.F. producibility without the anticoagulant activity by oral administration below a certain dosage. With the dose over 75 mg. per Kg. the prolongation of clotting time was seen. So the D. S., which contains the sulphur of around 18%, elevates the C. F. activity without the effect on the anticoagulant activity by the administration of 10 to 20 mg. per Kg. However, this is the result by the administration of single dose, and whether the clotting time is prolonged or not by the long term administration is another problem to be evaluated in future. According to the above fundamental experiment, the single dose of D. S. was given to 4 normal persons with the considerably high C. F. level in the plasma. As shown in Fig. 13, the administration of D. S. to arteriosclerotic patient by mouth every day resulted in the decrease in total cholesterol. Free cholesterol and the c/p ratio showed the same tendency. The D. S. used in this experiment contained the high level of sulphur and whether this D. S. has influence on the prolongation of clotting time by the long term administration or not is to be detected furthermore. Anyhow, the D. S. in the low molecular weight is obviously absorbed through the intestinal surface and I believe this makes the future of prophylaxis of arteriosclerosis hopeful.

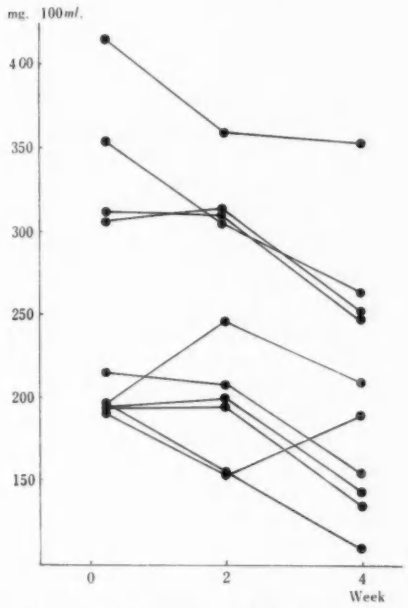

Fig. 13. Effect of oral administration of dextran sulphate (600 mg./day) on serum total cholesterol in men.

Summary

Although the etiology of arteriosclerosis in man is extremely complicated and it is difficult to see the whole view of this field, I believe that my co-workers and I have studied on the etiology and treatment for arteriosclerosis particularly in relation to Vitamin B_6 and lipemia clearing factor, and obtained favorable results in the prophylaxis of arteriosclerosis with dextran sulfate in relation to lipemia clearing factor.

(1) Pyridoxal phosphate may be relating to lipemia clearing factor as a co-enzyme of it.

(2) Vitamin B_6 deficiency was produced in the Japanese monkey and marked arteriosclerotic pictures were seen in these monkeys.

(3) Pyridoxal phosphate had the considerable effects on the treatment and particularly prophylaxis of experimental atherosclerosis in the rabbits fed lanolin.

(4) Pyridoxal phosphate had favorable effects on the treatment of arteriosclerosis in man with the improvement of fat metabolism and clinical symptoms, although additional cases should be studied before any conclusion is drawn.

(5) The dextran sulfate, which is low in the molecular weight and the sulphur content, is high in the clearing foctor activity, less toxic and very low in the anticoagulant activity.

(6) This kind of dextran sulfate showed sufficient result in the prophylaxis of experimental atherosclerosis in rabbits.

(7) This kind of dextran sulfate had a good influence on the lipid metabolism in aged person, although the method of administration should be further evaluated.

(8) The dextran sulfate of low molecular weight is absorbed through the intestinal surface and it may be possible to improve the fat metabolism in aged person by the oral administration, too.

Further study is needed in order to establish prophylaxis and treatment of arteriosclerosis in man.

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Radiographic Appearances of Pulmonary Complications in Cardiovascular Disease

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Tatsuya MOMOSE, M. D., *and* Makoto MURAO, M. D.

In the lung of cardiovascular patients, especially in cardiac decompensation, many pathological changes occur revealing various radiographic manifestations. It is important and necessary to get exact informations about these pulmonary changes though the interpretation of radiographic manifestations is often very difficult.

In the present paper, an attempt was made to evaluate radiographic findings in 23 autopsied cases comparing with pathological changes in the lung. Several cases with various pulmonary changes were demonstrated, and comparison between pathological changes and x-ray findings was described. Although further studies are necessary, the present results would offer an useful guide for the interpretation of radiographic findings in the lung.

IN spite of the recent progress in the interpretation of radiographic appearances in chest x-ray films of cardiovascular patients, an accurate diagnosis of pulmonary complications remains quite difficult. This is because of similar symptoms such as cough, sputum, hemoptysis or dyspnea in addition to similar radiographic shadows appearing in various pulmonary complications in cardiovascular patients.

The purpose of the present report is to evaluate radiographic findings of pathological changes of the lung in cardiac diseases. Some examples of autopsied cases were demonstrated and pathological changes in the lungs were described in comparison with the radiographic findings.

Materials

The materials of this study consist of 23 autopsied cases with cardiovascular diseases, which were admitted to Tokyo University Hospital during April, 1958 to March, 1960. They were 11 males and 12 females and the age varied from 14 to 64. The basic cardiovascular disease of these 23 cases was mitral

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valvular disease in 8, combined valvular disease in 7, aortic valvular disease in 3, coronary heart disease and myocardial infarction in 3, syphilitic heart disease in one and endomyocardial fibrosis in one.

Report of Cases

Some of the cases with interesting roentgenograms suitable for comparison with pathological findings were presented as follws:

Case 1. I. K., a 42-year-old woman, was admitted under the clinical diagnosis of mitral stenosis and insufficiency. The patient received a full digitalization. Two days after the hospitalization she suddenly died with an attack of convulsion.

A roentgenogram of the chest taken a day before the death showed well-outlined, increased lung markings and increased right hilar shadow (Fig. 1).

Postmortem examination revealed mitral stenosis and insufficiency. Microscopically, a marked pulmonary edema but no congestive discoloration was seen (Fig. 2).

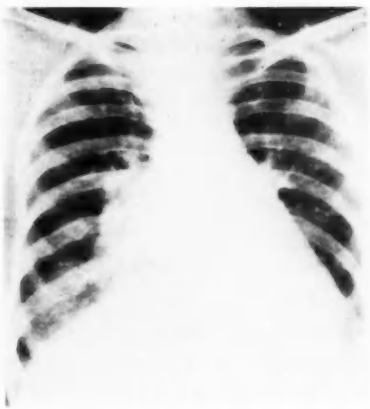

Fig. 1. Case 1. Increased lung markings and right hilar shadow.

Fig. 2. Marked pulmonary edema but no congestive discoloration.

Case 2. N. T., a 57-year-old man, was first diagnosed pulmonary tuberculosis because of general malaise, anorexia, remittent fever and x-ray findings of the chest. Streptomycin, however, was ineffective and an x-ray film revealed increase in pulmonary markings. On admission, aortic regurgitation was diagnosed by auscultation of the heart. Severe cough, a large amount of sleimy sputum, slight edema on extremities, tachycardia and tachypnea were seen. Dullness on percussion and moist râles were heard in lower lung field on both sides. Tubercle bacilli were not found in sputum. Dyspnea, venous engorgement and general edema became more marked. Oliguria, an elevation of blood NPN level and mental disturbance appeared in succession and he died

one month after the hospitalization.

The roentgenogram of the chest during the course showed increased hilar densities, widely scattered fine nodulations and densities near both hili and in the right lower lung (Fig. 3).

Autopsy revealed aortic valvular thromboendocarditis with aortic insufficiency and diffuse, greyish white induration of lungs (Fig. 4). Congestion with

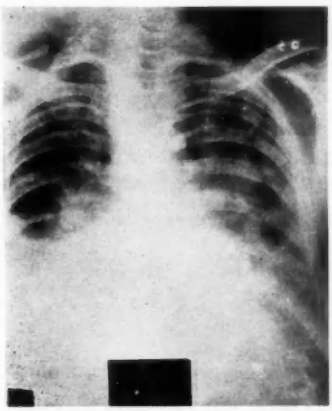

Fig. 3. Case 2. Increased hilar densities, widely scattered fine nodulations and densities near both hili and in the right lung base.

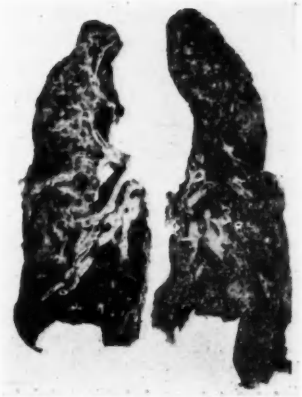

Fig. 4. Congestion with small hemorrhagic foci and small infarctions of diffuse, greyish white induration of the lung.

Fig. 5. Carnification with partial collagenization (arrow: carnification).

small hemorrhagic foci and small infarctions were also seen in the base of right upper and middle lobe. Microscopically, much intraalveolar exudate and carnification with partial collagenization in moderate degree of exudate were observed in indurated areas (Fig. 5). Effusion was found in the right (1,200 ml.)

and left (400 ml.) pleural space.

Case 3. Y. T., a 15-year-old girl, was admitted to the hospital with complaints of palpitation, edema, dyspnea, cough and blood-stained sputum. Combined valvular disease was diagnosed on admission. She had an episode of hemoptysis 3 months before the hospitalization. Anginal pain with dyspnea occurred suddenly 2 weeks after the hospitalization. Pericardial and pleural friction rub appeared 2 months after this episode of the pain and a diagnosis of complicated pleuropericarditis was made. In the following months, severe cough and hemoptysis re-occurred, and râles, fever and leukocytosis were found. She did not respond to the treatment and died.

In a film which was taken at the time of admission, homogenous opacities were recognized in the middle and lower field of the right lung. These shadows became confluent and larger after the occurrence of anginal pain (Fig. 6). Following the anginal pain, the chest findings were gradually absorbed

Fig. 6. Case 3. Homogenous opacities in the middle and lower field of the right lung.

Fig. 7. Brown induration with hemorrhagic infarction (arrow).

and became smaller and pneumonia or pulmonary infarction was suspected. Three weeks after the anginal pain, a wedge-shaped shadow appeared in the left upper-middle lung field in addition to the previously described shadows and was interpreted as left interlobar effusion.

Autopsy revealed rheumatic peri-, myo-, and endocarditis. Aortic insufficiency and mitral stenosis and insufficiency were proved. Several, up to 5×7 cm. sized pulmonary infarctions were found in the upper, middle and lower portion of the right lung and also in the left upper and lingual portion corresponding to the radiographic findings. Generalized edema, congestion and brown induration were observed in both lungs. Effusion was seen in the right (500 ml.) and left (1,300 ml.) pleural cavity. Hemorrhage around a number of miliary infarctions was seen in the right lung (Fig. 7).

Case 4. O.T., a 49-year-old man, was admitted to the hospital with complaints of nocturnal dyspnea, insomnia and edema of the lower extremities. An enlargement of the heart without any murmur was found. A tentative diagnosis of congestive heart failure due to sclerotic heart disease was made. Symptoms and signs of the cardiac decompensation became worse following an agonizing sensation in the chest occurred 3 months after the admission. Three weeks after this episode, dyspnea and hemoptysis occurred repeatedly and general condition became deteriorated and he died 6 months after the hospitalization.

X-ray films were taken frequently during the course of the hospitalization showing only constant pleural scar with a calcified linear shadow in the lower portion of the right lung. A chest film taken immediately after the episode of constrictive sensation showed a faint clouding near the hilum only (Fig. 8).

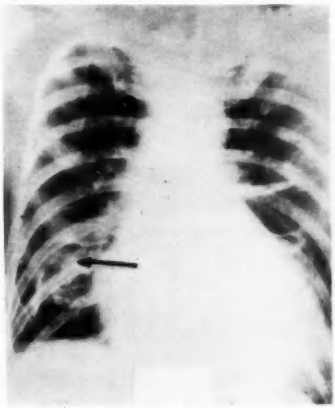

Fig. 8. Case 4. Pleural scar with a calcified longitudinal linear shadow in the right lung base and a faint clouding (arrow).

Fig. 9. Unevenly distributed congestion with brown induration and hemorrhagic infarctions. Necrotic cavity is found in some of infarctions (arrow: infarction).

Histological examination revealed endomyocardial fibrosis. In the lung, unevenly distributed congestion associated with hemmorrhage, brown induration and several hemorrhagic infarctions from 1.5 to 3 cm. in diameter were seen (Fig. 9). In some of these infarctions, necrotic cavity was found.

Case 5. N.A., a 59-year-old woman, was admitted to the hospital with complaints of paroxysmal nocturnal dyspnea and arrhythmia. She had had a history of edema on extremities and orthopnea for the past 4 years prior to the admission. Chronic glomerulonephritis and mitral insufficiency was diagnosed at that time and she received daily digitalis. On admission, she was orthopneic with cough and sputum. Pleural effusion on the left was proved by a chest film. Low urinary output, increased edema, elevation of NPN (150 mg./100ml.) and dyspnea gradually became worse in spite of the treatment and

she expired 5 months after the hospitalization.

Fig. 10, a chest film during the hospitalization, suggested an accumulation of pleural effusion in the left lower pleural cavity and septal lines were seen at the right lung base.

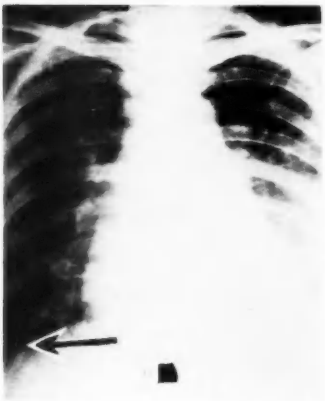

Fig. 10. Pleural effusion in the left side and septal lines near the right phrenicocostal angle (arrow: septal lines).

Autopsy revealed secondary contracted kidney and mitral stenosis and insufficiency. Pulmonary arteriosclerosis of a considerable degree and 1,500 ml. of fluid in the left pleural cavity were found. But there was no appreciable pathological changes corresponding to septal lines.

Comparison of Pathological and Radiographic Findings

As mentioned above, several pathological changes and several radiographic findings could be found simultaneously in the lungs of a cardiovascular patient, and it was impossible to correlate one radiographic finding with one pathological change. For trial, however, each pathological and radiographic finding was compared in spite of co-existence of other changes and the results were shown in Table I.

Macroscopic edema of the lungs was the commonest finding and it was frequently only the macroscopic change in lungs in cases that died suddenly by cardiovascular diseases. Edema was seen in 18 autopsied cases but there was only one case that could be diagnosed radiographically before death (case 2). Remaining 17 cases did not show any specific radiographic appearance of edema.

Manifest congestion was seen in 12 cases and was diagnosed radiographically in 10. Accordingly, in pulmonary congestion, the radiographic and pathological findings corresponded more frequently. Increased lung markings and scattered fine nodulations were main findings in these cases.

Table I. Comparison of Radiographic and Pathological Findings in the Lung in 23 Cardiovascular Patients

Pathological findings	Radiographic findings	Total	Lung markings			Hilar density			Disseminated nodulations		
			Normal	Increased		Normal	Increased		Normal	Distributed	
				Well-outlined	Ill-outlined		Slight	Marked		Over entire lung	Regionally
Congestion	+	12	2	7	3	6	2	4	3	6	3
	−	11	6	3	2	5	5	1	8	1	2
Edema*	+	6	2	3	1	3	3	0	3	1	2
	−	5	4	0	1	2	2	1	5	0	0
Brown induration	++	6	1	4	1	5	1	0	2	1	3
	+	9	5	1	3	3	3	3	5	3	1
	−	8	2	5	1	3	3	2	4	3	1
Pneumonia	+	7	1	2	4	1	2	4	0	3	4
	−	16	7	8	1	10	5	1	11	4	1
Tracheobronchitis	+	10	2	5	3	4	3	3	4	3	3
	−	13	6	5	2	7	4	2	7	4	2

* In order to exclude the shadows due to congestion, cases with edema but without high or moderate degree of congestion were selected here.

Hemorrhagic infarction of the lungs was seen in 6 cases and was diagnosed radiographically in 3 cases (case 3, 4 and in one case by a homogenous density in the right lung base associated with elevation of diaphragm). In the remaining 3 cases with infarction in which radiographic diagnosis of pulmonary infarction was not made: pleural effusion which overlapped the area of infarction was present in one, no corresponding radiographic appearances were seen in one and no x-ray film was taken after the occurrence of infarction in one case.

Gross hemorrhage was seen as a part of hemorrhagic infarction. Scattered small hemorrhagic foci were observed in 9 cases. But these small hemorrhagic foci usually showed no corresponding shadow.

Brown induration was marked in 6 cases and was slight in 9 cases. These changes usually did not give specific radiographic appearances as seen in Table I.

Tenacious, purulent material in bronchial tree was seen in 10 cases and scattered miliary foci of pneumonia were seen in 7 cases. Large focus of pneumonia was not found in these cases. Corresponding to these pathological findings, localized massive densities were not found but diffusely scattered and regionally disseminated nodulations were seen radiographically. Lung markings were often ill-defined in cases with pneumonia. Bronchitis was diagnosed rather by clinical symptoms and signs than by x-ray findings except in cases with combined bronchopneumonia.

Pleural effusion of some extent was an usual finding at autopsy and it was often seen radiographically.

As mentioned above, pathological changes sometimes coincided with clinical and x-ray diagnosis but often they did not.

Discussion

In cardiac decompensation, various pathological processes can occur in various portions of the lungs with various degrees. Radiographic examination is an important clue to know pulmonary involvements in cardiovascular diseases.

Generally accepted radiographic appearances of pulmonary congestion are increase in lung markings and in hilar shadows. Degree of congestion in the lungs of the autopsied cases was often pronounced in the lower portion due to influence of gravity, but it often varied at different areas according to anatomical factors, as seen in the case 4. Degree of radiographic change, however, did not always correspond to degree of pathological congestion. Diminution of markings caused by arterial constriction in some cases with pulmonary hypertension and exaggeration of markings caused by complication of bronchitis

and pneumonia probably modified radiographic appearances of congestion. According to Teschendorf,[1] besides "streifiges Lungenfeld", the second type of radiographic picture of pulmonary congestion is "geflecktes oder getüpfeltes Lungenfeld". He regarded the second type as a manifestation of crossing of engorged small vessels. The disseminated fine nodulations in the present article can be considered as shadows of such type. Increase in both lung markings and hilar densities and disseminated nodulations appeared often in combination and were all supposed to be the radiographic manifestation of pulmonary congestion.

Pulmonary hemosiderosis was first noted by Virchow in 1858. But its radiographic manifestation was described in recent years.[2] Hemosiderosis was caused by the hemorrhage following the congestion and it was observed as brown induration macroscopically. Microscopically, it was composed of thickening of alveolar septa and a mass of "heart failure cells" in alveolar spaces (Fig. 11). These cells were macrophages

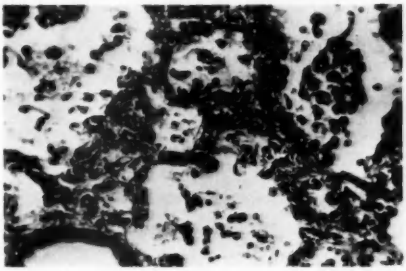

Fig. 11. Thickening of alveolar septa and a mass of "heart failure cells".

laden with hemosiderin and were said to be responsible for the radiographic "pulmonary nodules of hemosiderosis". But 2 points must be mentioned in the interpretation of such disseminated nodular shadows. One point is that when size of nodules was small and outline was hazed, these could not be differentiated accurately from "getüpfelt" nodulations of pulmonary congestion. The other is that brown induration was a quite common finding in autopsied cases with a long standing decompensation, but many of such cases did not show typical nodular shadows in a chest film as in the case 3. According to Oeser et al.,[3] hemosiderin does not absorb x-ray between 20 and 250 Kv. significantly. Therefore diagnostic value of radiographic "pulmonary hemosiderosis" is thought to be very limited.

Lungs of cardiovascular patients who died suddenly often showed pulmonary edema macroscopically as in the case 1. But radiographic

findings were not generally noticed in these cases, probably due to either very rapid formation of edema fluid before death or the possibility that thin fluid did not form a radiographic shadow. Lungs of patients who died after a long standing cardiac decompensation also showed pulmonary edema. But in such cases the induration and congestion were generally associated. Septal lines, which were said to be a manifestation of chronic pulmonary edema,[4] were seen rarely and chest films of these cases did not show any characteristic shadow representing pulmonary edema, except those shadows due to basic pathological changes such as induration and congestion. Macroscopically, edema was seen nearly evenly over entire lungs and this may be a reason why edema is not manifested radiographically. The case 2 showed nearly the same "bat's wing shadow" and same histologic picture of exudation and carnification as reported by Doniach[5] in cases treated with hexamethonium. Therefore the "bat's wing shadow", which has been said as a manifestation of acute pulmonary edema was thought to appear only in such specific type of edema or exudate, which was rich in protein content.

Pneumonia was a frequent finding associated with cardiac decompensation. Small foci of pneumonia were often observed but a gross pneumonic focus was not observed in cases which were treated with antibiotics. This fact may be important to consider about the radiographic appearances of complicated pneumonia in cardiovascular disease. In the case with localized density, rather atelectasis or pulmonary infarction may be suspected than pneumonia, as seen in the case 3. Regionally disseminated small nodulations were apt to appear due to a summation of small foci of cell-infiltration and might be one of the radiographic findings corresponding to bronchopneumonia. These nodulations were differentiated from other regional nodulations according to responsiveness to chemotherapy.

Pulmonary infarction gives sometimes no appreciable radiographic change. Normal chest film was found in 9.5% of pulmonary embolism of Short's report[6] and in 17% of Stein's report.[7] Some of the pathologically confirmed infarction showed no radiographic manifestation as shown in the case 4. In the case 3 and 4, localized homogenous density, linear shadow and in one case, even shadow suggesting pleural effusion and elevated diaphragm were possible radiographic manifestation of pulmonary infarction, as reported by Stein.[7] Thus radiographic appearances of pulmonary infarction are variable from case to case and clinical symptoms and signs should be considered for the diagnosis.

Septal lines were first described by Kerley[8] in 1933. Since then, a number of studies have been made. According to pathological study of Fleishner and Reiner,[9] septal lines were considered as radiographic

manifestation of thickening of interlobular septa. But the case 5 and another case with septal lines did not show corresponding pathological changes and the prevailing opinion was not ascertained.

In general, clinical and radiographic pulmonary findings appear and disappear in the course of cardiac decompensation and autopsy findings do not always correspond to radiographic findings. Besides disparity of time between 2 different examinations, there is also radiographic factor. Small, not dense, pathological changes, such as scattered, small hemorrhagic foci, do not appear radiographically. Marked pathological changes may not be seen radiographically unless there is some difference in translucency to x-ray between focus of changes and surroundings. Generalized edema and brown induration were such examples. Another unfavorable factor for getting an adequate film to read is instability of lungs due to tachycardia and shortness of breath. All these factors make the interpretation of radiographic findings very difficult.

In order to diagnose pulmonary complications exactly, to make a more adequate plan for the treatment and to presume prognosis, it would be important and necessary to make frequent radiologic examinations in the course of cardiovascular disease.

Summary and Conclusion

Radiographic manifestations of various pathological changes seen in the lungs were investigated in 23 autopsied cases of cardiovascular diseases.

The results lead to the following conclusions:

(1) Increase in lung markings, increased hilar shadows and diffusely scattered nodulations were radiographic manifestations of pulmonary congestion. Pulmonary edema commonly seen in cardiac decompensation was usually missed in radiographic examination.

(2) Brown induration was a frequent finding in the lungs of autopsied cases but shadows of so-called "pulmonary hemosiderosis" was not generally seen in such cases.

(3) Septal lines seen in 2 autopsied cases did not show corresponding pathological changes.

(4) Pulmonary infarction sometimes did not show any radiographic finding. Homogenous density, linear shadow, shadows suggesting pleural effusion and elevation of diaphragm were possible manifestations of infarction.

(5) Pneumonia appeared generally as miliary foci both pathologically and radiographically.

Acknowledgement

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Successive Measurements of Cardiac Output by External Monitoring Using Digital Counter and Automatic Digital Printer

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The selection of a calibration time for an external radioisotope determination of cardiac output is discussed with respect to obtaining values which agreed well with those of direct arterial sampling technique. Placement of external detector was also examined using 2 scintillation head with different collimators and focusing angulations simultaneously. External cardiac output demonstrated higher value by conventional 10 minute calibration method than 5 to 3 minute ones, and the values calibrated at 5 minute after RISA injection were in most reasonable agreement with those of direct value, and the authors recommended the left cardiac apex monitoring with 5 minute calibration as the standard method. Examples of successive determination of cardiac output before and aftere aminophylline injection are illustrated, along with a presentation of newly deviced digital counter and automatic digital printer which are convenient for such serial determination. Response to the aminophylline was different between the normal individuals and the patients with heart failure. The utilization of the method presented may add an important clinical examination of cardiac function.

THE methods most widely used to measure cardiac output are based either on the Fick or on the Stewart-Hamilton principle.

Fick method requires cardiac catheterization and can rarely be performed more than once on the same subject. The conventional Hamilton method using T-1824 as the indicator can also rarely be performed more than twice on the same subject because of its skin coloration. However, successive determinations of cardiac output at short

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intervals are requested to examine the cardiac function more clearly. In the original Hamilton method,[1] after an indicator is injected intravenously, the arterial concentration of the indicator should be measured as a function of time by drawing the blood samples every one to 2 seconds for about one minute. Then the feasibility of estimating cardiac output from precordial dilution curve using radioactive materials was first suggested by Prinzmetal and coworkers.[2] Subsequently, Shipley and associates[3] gave estimates of blood flow, though the values obtained by them were twice as great as those of conventional methods, using scintillation equipment and wide angle collimator to record radiocardiogram. In Japan, Ueda and cowokers[4] have also used radiocardiography in 1954, using radioactive sodium (^{24}Na), for the study of pulmonary circulation. Huff[5] and MacIntyre, Pritchard[6] and his coworkers have made refinements in the technique of the external determination of cardiac output, using radioactive human serum albumin (RISA) tagged with ^{131}I, and gave estimates of flow which approximated those obtained by other methods. Thus the usefulness of an isotope dilution technique with external body counting in determination of cardiac output has been demonstrated for several years by many other authors.[7]-[13] In Japan, appraisal of this method has been carried out in our radioisotope laboratory at first,[14] and this test is now going to be used in many hospitals and radioisotope laboratories.

The following study was undertaken to evaluate 3 calibration times and different collimators by comparing the precordial method with direct arterial sampling method, and to extend the method to the successive measurements within short intervals using newly deviced digital recording apparatus of our own.

Materials and Methods

Equipment:—

The scintillation counter used in this study has been described previously.[15] As shown in block diagram of Fig. 1 and Fig. 2, 2 detectors and 2 scalers were used and the changes in radioactivity were recorded simultaneously. Both the recording system using galvanometer-type pen recorder[15] and the other system using new apparatus were used in this study.

Fig. 3 shows the dimensions and efficiency characteristics of 3 collimators tested. Two scintillation heads placed on the left and right precordium respectively were used simultaneously throughout the studies. One scintillation head was focused over the apex of the heart and the other over the right sternal border at the 4th intercostal space, and both of these methods were examined making a different focusing angulation against the horizontal plane, so that left and right ventricles should be viewed predominantly (Fig. 2B, Fig. 10).

Digital Counter and Automatic Digital Printer:—

Pulses from the 2 photomultiplier pass through the pulse height analyzer

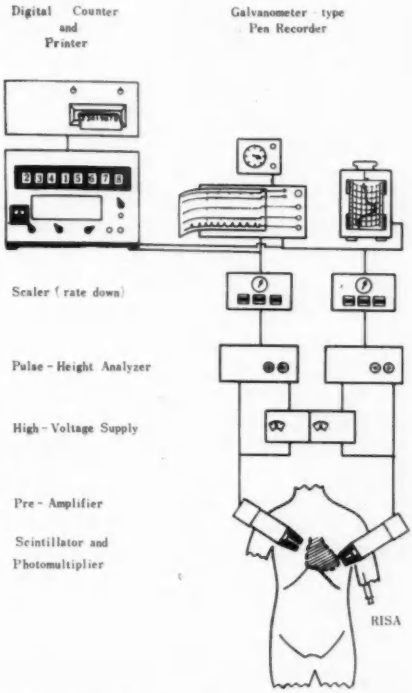
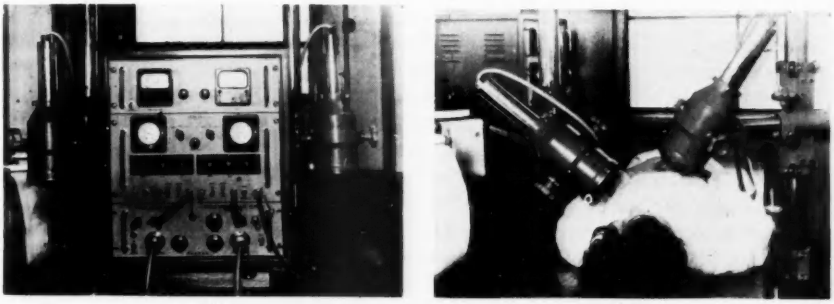


Fig. 1. The arrangement of apparatus for determination of radioactivity of the cardiac region after intravenous injection of ^{131}I albumin.



(A) (B)

Fig. 2. A: Scintillation counters. B: Position of patient in laboratory and the detectors placed over the right and left precordium at the same time. For correlation, a peripheral artery was used as the site of drawing samples.

to eliminate the low energy scattering γ rays, and the remainders were recorded automatically every one second by digital counter and automatic digital printer.

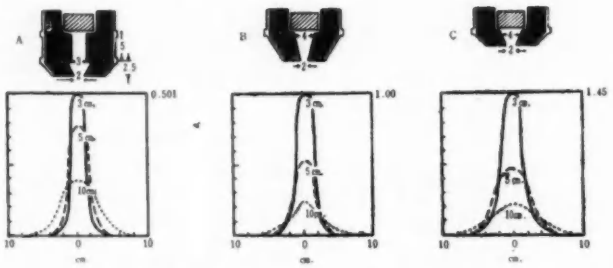
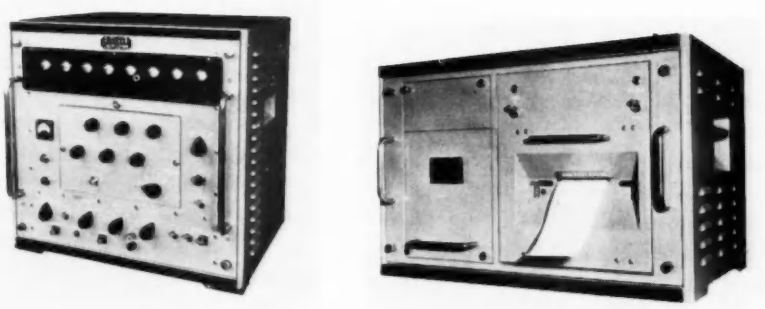


Fig. 3. Dimensions and efficiency characteristics of 3 types collimators representing A (high), B (middle) and C (moderate) collimation. Every collimator was used in the correlation study and B collimator is utilized now for the routine standard study.



(A) (B)

Fig. 4. A: Digital counter.
B: Automatic digital printer.

This machine was deviced and produced through Oki Electric Industry Co. in Japan in cooperation with our laboratory. The photograph and the block diagram of this recorder are illustrated in Fig. 4 and 5. The circuit involved in this recorder performs several functions. It counts the number of pulses entered during the gate is open, and prints it automatically in succeeding short period. Four sets are arranged for the purpose of several radioisotope study (Table I). The first set of Table I was used in this study and radioactivity could be recorded automatically every one second successively as illustrated in Fig. 5 B. Pulses are counted for 0.6 second when the gate is open, representing one hundredth counting rate per minute, and then automatic digital printer makes a rapid printing within following 0.4 second. When a high background is present or successive examination is needed, the operation of the complement selecter can remove the influence of these elevated counting rate upon the final reading by shifting the starting level down below zero, and one can read

directly the necessary counting rate which is free from background counts. This operation is also described in block form in Fig. 5 A. When counting rate is too high and exceeds the full scale of digital counter, conventional scaler placed before this counter (Fig. 1), consisting of 3 EIT tubes, is used to transmit

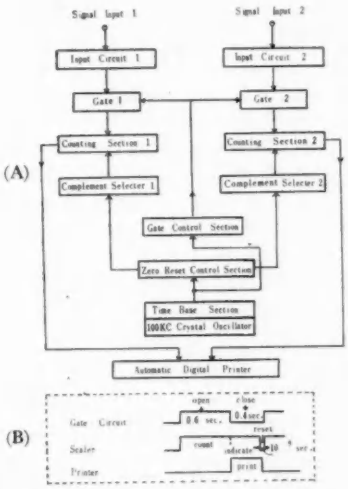


Fig. 5. (A) Block diagram of digital counter and automatic digital printer deviced in our laboratory and produced through Oki Electric Industry Co. in Japan.
(B) Mechanism of the function of this circuit.

pulses after the collection of predetermined number 10, 100 or 1000 counts, thus operating the function as a rate down unit. If the changes in the radioactivity are prerecorded on the tape and played directly into the input terminal of

Table I.

	Gate open (counting)	Gate closed (printing and resetting)
1	0.6 sec.	0.4 sec.
2	1.0 sec.	1.0 sec.
3	30.0 sec.	0.5 sec.
4	60.0 sec.	0.5 sec.

this digital counter at one tenth slower speed, every 0.1 second counting of the original phenomena is also possible by this machine and even the rapidly changing dilution process by every cardiac cycle can be recorded.[16]

Materials:—

The materials consisted of 37 cases, including 10 normal subjects, 13 patients

with heart failure, 10 with liver cirrhosis 2 with anemia and 2 with hyperthyroidism.

Procedure :—

The examination was carried out in a fasting and resting state in a supine position. Two scintillation heads with different collimators and various focusing angulations were focused respectively over the right and left side of the heart simultaneously as described above. The ^{131}I labeled human serum albumin containing 20 to 50μc of radioactivity was rapidly injected into the cubital vein and the dilution curves were recorded over the precordial region. At the same time, the blood samples were continuously drawn from the brachial or femoral artery in all cases and the cardiac output was directly calculated by Stewart-Hamilton method. The values obtained by this direct technique were compared with those of external technique in each case (Fig. 6). Blood samples

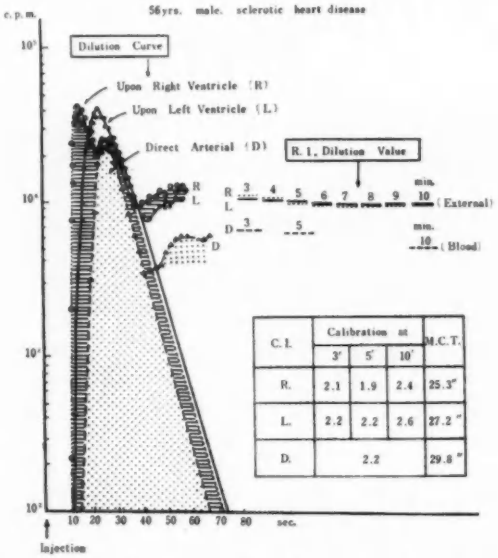


Fig. 6. Tracing from a case with sclerotic heart disease by two external counting over the left and right heart region and by direct arterial sampling method. Note that decrease in the external dilution values does not run parallel with the decrease in the blood dilution ones and produces high cardiac output if calibration is made at 10 min.

were also taken at 3, 5 and 10 min. after the injection in order to calibrate the external dilution value against the blood dilution value. Cardiac output by external method was calculated at each point of these 3 calibration periods by using the equation (3) in Fig. 7 and was compared with the direct values.

Reproducibility of this method was also examined on subjects in a resting

state without giving any medications, then the effect of aminophylline was studied in 6 cases including 3 cases of heart failure. The measurements of cardiac

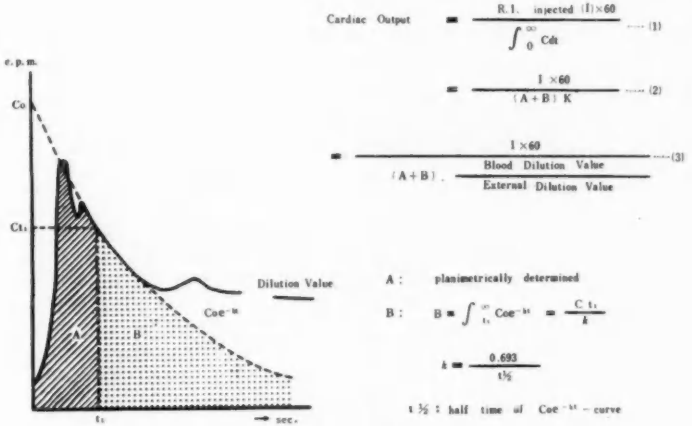

Cardiac Output = R.I. injected (I)×60 / ∫₀^∞ Cdt ---- (1)
= I ×60 / (A+B) K ---- (2)
= I ×60 / (A+B) . Blood Dilution Value / External Dilution Value ----(3)
c.p.m.
Co
Ct₁
Dilution Value
Coe^-kt
A
B
t₁
sec.
A : planimetrically determined
B : B = ∫_{t₁}^∞ Coe^-kt = C.t₁ / k
k = 0.693 / t½
t½ : half time of Coe^-kt - curve

Fig. 7. This formula derived originally from MacIntyre and co-workers and Huff et al. Modification was made as follows: the ratio of blood dilution value to external dilution value was used in our study instead of total blood volume and final dilution value of MacIntere et al., because in our study calibration was performed at various periods before complete mixing of R. I. was reached.

output were performed repeatedly one to 3 times within 15 to 25 minutes after the intravenous injection of 250 mg. of aminophylline.

Results

1) Analysis of calibration time

In external counting method, both the right and left external dilution values were caribrated at 3, 5 and 10 minutes after the injection of RISA using blood samples taken at the same time. Thus 6 calculated values were obtained for each determination. Variations of calculated cardiac index against 3 calibration periods are illustrated in Fig. 8. In control group, most cases show larger values by 10 minute calibration method than those obtained by 3 or 5 minute method. The percent changes (mean value of these cases) are shown below. Such tendency was not found in cases of heart failure, and the mean values at these 3 caribration times were almost equal to each other. In Fig. 9, mean values of the variation of blood and external dilution values at each calibration time of these cases are shown as percent change taking 5 minute value as 100%. It can be presumed that if a relatively complete mixing is reached in an early stage after RISA injection, the ratio

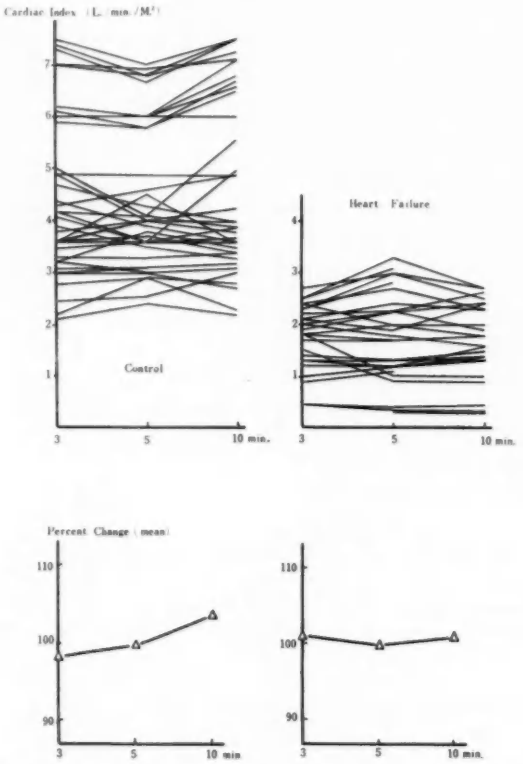


Fig. 8. Variations of calculated cardiac index by different calibration period (3,5 or 10 min.). Note that cardiac index caribrated at 10 minute showed higher value than 3 or 5 minute's one.

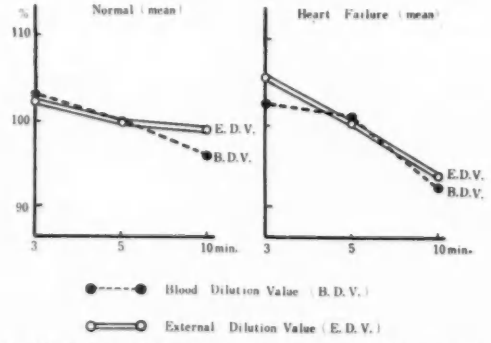


Fig. 9. Variations of both external and blood dilution value against calibration time. Note that external dilution value of control cases does not decrease parallel with the blood dilution value.

of the blood dilution value to the external dilution value should be constant throughout the following period. Our observations, however, demonstrated that in the period from 5 to 10 minutes the external dilution value showed little decrease, or in some case it even showed an increase, whereas the blood dilution value continued to decrease (Fig. 9). On the other hand, in heart failure both of them showed a sharp and parallel decrease in this period (Fig. 9). Comparisons of the values by the external method at different calibration times with those of the direct arterial method are presented in Table II. As seen in Table II, the

Table II. Deviation of External Method Value from Direct Method Value*

Calibration period (min.)	3	5	10
Left external (L./min./M²)	+0.21 ±0.39	**+0.16** ±0.22**	+0.44 ±0.56
Right external (L./min./M²)	+0.32 ±0.24	+0.23 ±0.33	+0.55 ±0.32

* The data obtained from 13 cases, including 9 normals and 4 cardiacs

** Significant difference compared to 10 min. value

external value of cardiac index showed positive systemic deviation to the direct value, however, the values calibrated at 5 minutes indicated minimal deviation and well correlated to the direct value.

2) Collimation and placement of the scintillation head

Three tapercone collimators shown in Fig. 3 were used with different

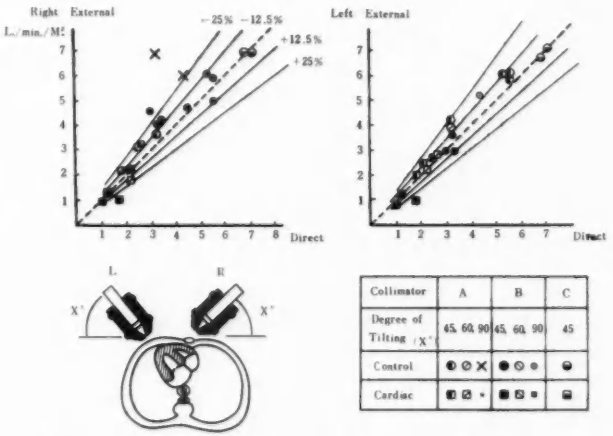


Fig. 10. Comparison of cardiac index values calculated from an arterial sampling curve and both right and left external heart curve calibrated at 5 minutes after injection.

focusing angulations against the chest wall. Comparisons of both the left and right precordial method calibrated at 5 minutes with the direct arterial method are illustrated in Fig. 10.

When the narrow collimator A with small inclination was used for the right external technique, the values obtained were too large since marked increase in external dilution values appeared. However, if the moderate collimator with adequate inclination was employed and a selective placement on the cardiac region was made, the obtained values were in reasonable agreement with those of the direct ones. When the left external technique was employed, where the collimator was focused over the cardiac apex, there was no difficulty in selection of the inclination of collimator and the values obtained by either collimator A or B well agreed with those of direct technique. The collimator C with minimal collimation in our series, though in only few cases, also gave satisfactory results. In cases with severe heart failure, it gave lower values as compared with direct technique.

From the above mentioned results, our standard technique for the external measurement of cardiac output was decided as follows: Collimator B is employed, which is focused over the cardiac apex with an inclination of 30 to 40 degrees against the horizontal plane and the calibration is made at 5 minutes after the injection.

3) Cardiac output in various diseases

The values of cardiac index determined by our method was 3.7 ± 0.3 L./min. in normal control, 1.8 ± 0.2 L./min. in heart failure, and

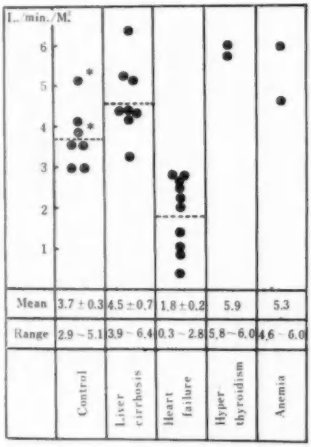


Fig. 11. Cardiac index determined by left precordial method calibrated at 5 minutes after injection.
Note: * These 2 cases were calibrated at 10 min.

4.5±0.7 L./min. in liver cirrhosis. The values in hyperthyroidism or anemia were larger than normal control (Fig. 11).

4) Successive determination of cardiac output

One of the advantages of this radioisotope external method for the measurement of cardiac output is that several determinations can be performed repeatedly within a short time. When the successive determinations of cardiac output were carried out on the same subjects, relatively

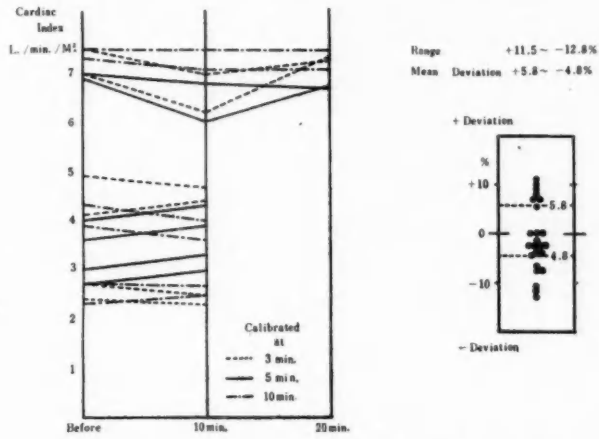


Fig. 12. Reproducibility in successive determinations.

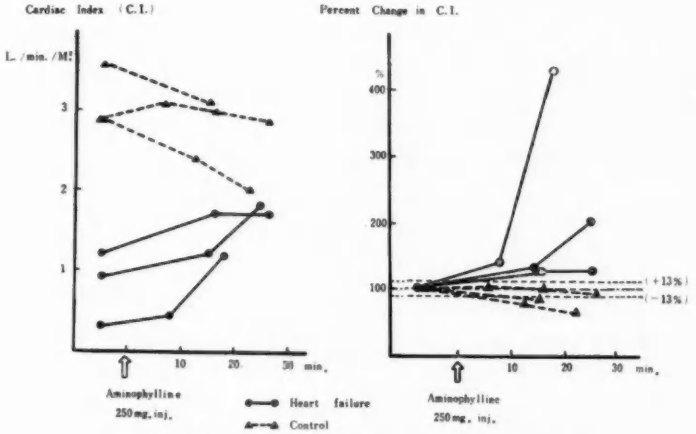


Fig. 13. Effect of aminophylline upon cardiac output.

constant values were obtained with an average deviation between +5.8% and −4.8% and the maximal deviation within ±13% (Fig. 12). Thus

the changes in cardiac output over ±13% were considered to be significant.

In a series of determination in 6 cases consisting of 3 normal subjects and 3 cases of heart failure, 250 mg. of aminophylline was injected intravenously, and the measurement of cardiac output was repeated 2 or 3 times within 15 to 25 minutes. Aminophylline produced little or no change in cardiac output in normal control but in patients with heart failure it caused an increase in cardiac output after about 10 minutes (Fig. 13). This increase was especially remarkable in cases whose cardiac

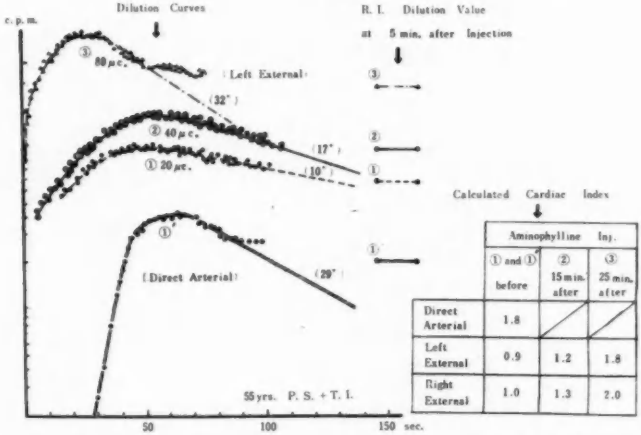


Fig. 14. Successive measurement of cardiac output after aminophylline injection in the cases of heart failure.

Note the gradual increase in cardiac output and shortening of circulation time after the injection. Marked discrepancy between the values determined by external method and direct arterial sampling method was also observed.

output was low before the administration of the drug. As shown in Fig. 14, following the injection of aminophylline in cases of heart failure, the appearance time and the disappearance time gradually shortened and the slope of the final extrapolated curve became steep, resulting in a shortening of the circulation time and an increase in cardiac output.

Discussion

For the calibration, the ***in vivo*** blood has to be drawn at the time when the external detector records the radioactivity of just identical mixing pool observed by the detector during the primary circulation curve. Calibration has been performed by many authors[5)-13)] at 10 to 15 minutes after radioisotope injection when relative equilibrium has been reached.

According to MacIntyre and collaborators,[6)-e] if the counting rate at this time shows lower value than that immediately following the primary curve, it is considered that the calibration requirements are satisfied and that the observed pool had no later component that comes into the region after the period of primary circulation. However, the authors examined the variation of both in vivo blood and external dilution values successively and pointed out the fact that the conventional calibration time must be improved to obtain the cardiac output values which show better correlation with direct ones.

As indicated above, external cardiac output demonstrated higher value by conventional 10 minute calibration method than 3 to 5 minute ones in control cases, and the values calibrated at 5 minute after injection were in most reasonable agreement with those of direct method. The reason of this was also investigated, since a marked discrepancy between the decrease in external dilution value and that in blood dilution value was observed at 10 minutes. In other words, this is due to some smaller later component which comes into the region under the scintillation head, probably participation of some slower mixing phases, gradual increase in whole body background and so on. These slower mixing phases have been neglected or considered to be insignificant by other authors. In our opinion, the observed increase in external dilution value can be attributed mainly to the later appearance of radioactive isotope within the intervening tissue under the scintillation head. The lack of this increase in cases of heart failure can be explained by an assumption that the mixing process is still progressing in this period resulting in such a marked decrease in the dilution value that the increase due to the activity within the tissue is too small to manifest itself. However, as in control, marked discrepancy between these 2 dilution values appeared in cases of heart failure 15 to 30 minutes after the injection and supported validity of our assumption.

Thus the calibration should be made after the fairly complete mixing is reached but before the increase in external dilution value takes place. According to our data, 5 minute calibration seems to be most appropriate from this point of view, and the values calibrated at 5 minute were in best agreement with those obtained by direct method (Fig. 9).

As for the placement of scintillation head, the authors recommended the left cardiac apex monitoring with 5 minute calibration as the standard method. By this method, the difficulty to decide the suitable position and angulation could be minimized and the obtained values correlated well with those of direct ones. MacIntyre and associates informed[6)-f] that the left heart focusing method was recommended, because abnormally high cardiac output may be recorded by right heart focusing method owing to

possible poor mixing of the radioactive tracer. The advantages of the left precordial method over the right one were also pointed out in cases of heart failure or obesity, since these cases are frequently associated with left heart enlargement.[6)-f] Zipf[8)-a] and collaborators focused external detector on the middle of cardiac shadow, and Huff,[5)] Schreiner[9)] and their associates upon the aortic arch with narrow collimation. In principle, external monitoring of head curve could be used for measuring cardiac output by the injection method, but Feer et al.[5)] pointed out that it was not very successful because of the rather high standard deviation of the result (25%).

Our findings confirmed and extended the observations of Huff,[5)] Mac Intyre[6)] and their coworkers who found good correlations of flow rates when compared to measurement made by Fick and direct arterial Hamilton determination. In addition, simultaneous direct arterial and our external cardiac output determination showed no significant differences. These findings could be attributed to adequate placement, collimation of the detector and improved 5 minute calibration method.

The validity and applicability of this method is further supported by many authors. The difference between the values measured by external method and direct arterial method was within $\pm 9\%$ by MacIntyre[6)-f,g] and within $\pm 10\%$ by Feer.[11)] An average deviation of the external values from the Fick values was within 10% (Seldon[17)]) or $+8.7\%$ (Sharpe[11)]), and no systemic difference could be found by Schreiner[9)] between these 2 methods.

However, the principal disadvantage of this method is that the applicability of this method is sometimes limited in the presence of severe decompensated heart failure. As shown in Fig. 14, both the cardiac output and the slope of the dilution curve obtained by the direct technique were larger than those by the external technique. Under such circumstances the composite precordial curve seems to be particularly unreliable if one wants to obtain accurate absolute flow rate by this method. Relatively large values in direct technique are attributed to the fact that the calculation is based on the dilution curve of rapid circulating space. On the other hand, the dilution curve of external technique is a complicated one since it is influenced by many factors such as the increase in residual volume of the heart and transit of the isotope into the slow circulating space within the lung. In addition, the difficulty in separation of the recirculation wave and the delay in the clearance slope of the external dilution curve are thought to make the calculated values of cardiac output smaller. However, this method still has advantages if successive determinations are performed in such cases of heart failure as illustrated in Fig. 14.

We cannot overestimate the usefulness of a single determination of

cardiac output to distinguish border lines of cardiac disease, since existing standard of the cardiac output at resting state shows widely scattered ranges and many patients fall within the normal limits. The successive measurements of cardiac output by external monitoring may add another important clinical evaluation of cardiac function. Authors have explored the clinical applicability of a cardiac output test in response to aminophylline injection with external monitoring in this report. Zipf and collaborates[8) a,b] performed serial cardiac output determinations in response to the load imposed by the Master test and Mahoney[10)] suggested the usefulness of this method to diagnose the prognosis of heart disease by measuring successively the changes of cardiac reserve, especially in cases of myocardial infarction. Thus this technique has the potential of providing important additional information on the dynamic pump function of the heart in response to drugs, exercise and postural change, and these informations may be of significant value in the clinical examination of cardiac function.

Summary and Conclusion

(1) An improved apparatus for the external counting of the radioactive isotope was deviced, which enabled us to make a digital recording and printing with an increased accuracy, without a time delay and has special device to remove background counting rate automatically from the final reading. This apparatus was proved to be adequate for the exact external measurement of the cardiac output as well as for the successive determinations.

(2) Several fundamental problems concerning the external measurements of cardiac output in man were investigated. The placement of the collimator focusing directly over the apex of the heart and the calibration at 5 minute were found to be most appropriate.

(3) Taking these results into account, the cardiac output was determined by means of external technique in 37 cases including normal subjects and patients with heart failure, liver cirrhosis, anemia and hyperthyroidism.

(4) The values obtained were in reasonable agreement with those of direct arterial sampling technique except in cases of severe heart failure.

(5) Successive determinations of cardiac output were performed in 6 cases before and after the intravenous injection of aminophylline. The response to the drug was different between the normal subjects and the patients with heart failure.

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Studies on Ventilatory Mechanics in Cardiacs and in Patients with Pulmonary Diseases

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In evaluating the ventilatory disturbances in cardio-pulmonary diseases, mechanical studies or washout of nitrogen by oxygen inhalation have been used. Authors studied the ventilatory disturbances in cardiacs and in patients with pulmonary diseases through the 2 methods; one is mechanical studies including measurements of effective compliance, flow resistance, viscous work and total work of the lungs at different respiratory rate, and the other is measurement of gradients of alveolar N_2 fraction in single breath analysis. The results obtained indicate that the ventilatory disorder in cardiacs and in patients with pulmonary fibrosis differ markedly from those in patients with emphysema. In addition, authors tried to elucidate the essential nature of ventilatory disorders in those diseases on physiological basis.

A number of reports have appeared during recent years on the changes of the physical properties of the lung in cardio-pulmonary diseases.[1)-25)] High flow resistance and dependency of compliance on respiratory rate were observed in patients with emphysema, while the decreased compliances were noted in cardiacs and in patients with pulmonary fibrosis. Previous authors measured such mechanical values as compliance, flow resistance and work of breathing in patients with emphysema, in cardiacs or in patients with pulmonary fibrosis separately. However, the attempt to secure the essential nature of each ventilatory disorders in comparing these mechanical values is not many. Moreover, the reports of measurement of uneven alveolar ventilation during a single breath of oxygen as well as the mechanical values in various respiratory rate, for the estimation of uneven distribution of ventilation in the lungs are not found.

The purpose of this study is to secure the physical characteristics of the lungs in cardiacs, in patients with emphysema and in patients with pulmonary fibrosis by comparing each other through the 2 methods; one

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is the mechanical study including the estimation of mechanical values at different respiratory rate, and the other one is the estimation of the gradient of alveolar N_2 fraction by single breath analysis.

Materials and Methods

Materials consisted of 14 normal subjects (Table I), 14 cardiacs, 11 patients with chronic pulmonary emphysema and 6 with pulmonary fibrosis of different origin (Table II). At the time of study, every cardiac had some degree of respiratory distress on regular walking.

Table I. Physical Properties of the Lung in Normal Subject

Subject	Age (yrs)	Sex	Height (cm.)	VC (L.)	Compliance (L./cm. H_2O)	Flow resistance (cm. H_2O/L./sec.)	Work of breathing (Gm. M./L.)	
							Viscous	Total
T. S.	27	M	160	—	0.15	1.43	15.0	36.4
E. Y.	28	M	168	—	0.28	1.44	16.8	22.9
M. M.	40	M	162	—	0.17	1.83	14.3	26.2
M. Y.	52	F	150	2.33	0.15	3.43	28.7	31.2
S. K.	42	M	157	2.70	0.16	2.35	15.0	19.7
Y. K.	31	M	163	2.95	0.17	3.36	20.9	22.3
Y. H.	39	M	160	3.04	0.14	1.51	13.2	25.9
M. T.	26	M	160	3.14	0.17	3.97	25.2	26.7
M. O.	25	M	168	4.27	0.18	2.79	18.6	18.7
Y. T.	19	M	173	4.43	0.16	3.24	25.4	27.6
H. H.	17	M	—	—	0.14	2.21	17.8	34.6
K. S.	29	M	—	—	0.16	0.77	4.04	9.23
H. S.	54	M	147	3.43	0.11	—	14.4	16.3
F. H.	34	M	169	—	0.17	1.14	10.1	22.4
Mean					0.17	2.26	17.1	24.3
S. D.					0.049	0.31	6.0	7.0

Effective compliance, viscous work and total work done on the lungs were measured and calculated by the same method as that previously reported,[25] but viscous resistance was calculated as average resistance for inspiration and expiration following the method of Frank et al.[26] Effective compliance and mechanical works were measured at eupnea, at voluntary bradypnea and at voluntary tachypnea.

Our method for detection of uneven alveolar ventilation during a single breath of oxygen is principally that described by Fowler[27] and Comroe.[28] Actual method was the same as that described by Koike.[29]

Results

a) Normal subject

Table II. Lung Volumes and Ventilatory Functions in Patients with Cardio-pulmonary Diseases

Subject	Age (yrs)	Sex	Height (cm.)	Diseases	FRC (L.)	RV (L.)	TLC (L.)	RV/TLC ×100	VC (L.)		MBC (L./min.)		MMF (L./sec.)	Gradient of alveolar N_2 fraction
									observed	predicted	observed	predicted		
R. S.	48	M	148	Mitral stenosis and insufficiency	1.20	0.83	3.00	27.6	2.00	3.30	72.6	81.0	2.73	
Sh. K.	31	F	156	〃	1.61	1.24	3.48	35.6	2.16	2.90	68.0	83.0	—	
K. F.	40	M	159	Mitral stenosis	2.46	1.52	4.42	34.4	2.91	3.70	61.7	95.0	1.27	
K. N.	18	F	154	Mitral stenosis and insufficiency	—	—	—	—	—	—	—	—	—	
Ya. H.	17	F	156	〃	1.61	1.06	2.55	41.6	1.33	3.12	32.1	84.0	0.65	0.5
Ma. M.	15	M	155	〃	2.22	1.19	3.60	33.1	2.78	3.78	104.0	110.0	1.48	0.4
T. M.	56	F	151	〃	0.91	0.82	1.69	48.5	1.25	2.40	21.4	64.0	1.25	0
T. O.	43	M	167	〃	3.30	2.45	4.66	47.4	1.88	3.88	35.6	95.0	0.53	2.8
M. S.	43	M	167	〃	2.90	1.73	4.21	41.4	2.66	3.90	99.3	98.0	2.06	0.2
S. S.	59	F	144	〃	1.78	1.58	2.88	55.0	0.93	2.20	25.6	50.0	0.56	0
S. T.	29	M	190	〃	—	—	—	—	—	—	—	—	—	
Sa. T.	15	M	159	〃	—	—	—	—	—	—	—	—	—	
Mi. S.	20	M	172	〃	3.61	2.14	6.32	33.6	4.40	4.41	96.7	130.0	2.71	0.8
Ry. K.	26	M	164	Mitral stenosis	2.24	1.04	4.07	25.6	3.43	4.05	72.0	106.0	3.79	0
R. K.	64	M	154	Emphysema	3.96	3.10	4.42	68.0	2.28	3.16	65.7	70.0	—	
T. T.	43	M	166	〃	3.92	2.38	6.88	34.6	4.18	3.79	86.8	91.0	1.60	
M. H.	43	M	164	〃	5.41	4.16	7.40	56.2	3.00	3.50	34.8	79.0	—	
M. K.	47	M	164	〃	3.47	2.63	5.47	48.1	2.72	3.68	56.6	96.0	1.09	5.6
T. K.	41	F	147	〃	2.77	2.42	3.85	62.9	1.40	2.60	37.0	65.0	1.22	7.8
A. N.	66	F	145	Emphysema with cor pulmonale	2.59	1.81	3.23	73.5	1.26	2.19	19.9	41.4	0.19	14.0
T. T.	67	M	154	Emphysema	3.43	2.34	5.10	46.0	2.58	3.10	64.5	69.5	1.01	
Mu. T.	68	F	144	〃	1.75	1.42	2.51	56.6	0.93	2.15	21.8	45.0	—	
I. S.	52	M	154	〃	3.09	2.38	4.81	49.5	1.86	3.15	25.2	80.0	—	12.0
Tt. O.	58	F	148	〃	3.69	3.10	4.61	66.8	1.34	2.35	11.7	55.0	0.29	
T. H.	41	M	147	〃	3.78	3.00	4.64	64.7	1.56	2.34	18.1	54.0	0.21	
N. A.	39	M	176	Berylliosis	3.18	1.80	5.95	30.3	3.96	4.45	99.0	131.0	0.85	
To. M.	21	M	153	〃	1.05	0.82	1.81	45.3	0.86	3.85	44.4	105.0	0.28	4.0
K. T.	61	M	176	Pulmonary fibrosis	2.11	1.44	4.11	35.0	2.45	3.70	48.4	78.0	—	6.0
H. I.	59	F	155	Radiation fibrosis	0.98	0.70	2.45	28.6	1.75	2.57	49.1	68.0	1.01	0.81
H. T.	63	F	149	Diffuse interstitial fibrosis	2.59	1.74	4.07	42.8	2.33	3.11	75.4	59.0	1.27	9.0
K. O.	47	M	166	〃	1.77	1.11	3.05	36.5	1.50	3.75	56.9	114.0	1.11	

Mechanical values in resting respiration (Table I)

The average value for compliance was 0.17L./cm. H_2O (S. D. 0.049). The average value for flow resistance was 2.26 cm. H_2O/L./sec. (S. D. 0.31) and 9 subjects out of 14 showed values below 3 cm. H_2O/L./sec. The average value of viscous works was 17.1 Gm. M./L. (S. D. 6.0), and the mean value of total works was 24.3 Gm. M./L. (S. D. 7.0).

Table III. Values for Ventilatory Mechanics of the Lungs in Patients with Cardio-pulmonary Diseases

Subject	Compliance (L./cm. H_2O)	Average resistance (cm. H_2O/L./sec.)	Work of breathing in resting (Gm. M./L.)	
			Viscous	Total
Cardiacs				
R. S.	0.087	2.78	13.7	31.9
Sh. K.	0.084	5.10	44.8	64.5
K. F.	0.092	5.43	67.0	91.7
K. N.	0.11	5.09	40.9	57.2
Ya. H.	0.055	3.44	21.5	46.9
Ma. M.	0.066	5.58	41.5	57.2
T. M.	0.046	7.60	55.2	73.0
T. O.	0.067	7.40	44.4	58.4
M. S.	0.034	2.90	—	—
S. S.	0.042	8.40	53.6	66.4
S. T.	0.16	0.61	—	—
Sa. T.	0.17	1.75	13.3	18.6
M. S.	0.22	3.45	28.4	31.8
Ry. K.	0.10	2.30	24.6	39.3
Mean	0.096	4.42	37.4	53.1
S. D.	0.052	2.38	16.3	19.5
Chronic Pulmonary Emphysema				
R. K.	0.13	6.07	33.0	51.7
T. T.	0.25	2.72	35.4	40.2
M. H.	0.073	11.2	60.5	61.0
M. K.	0.34	6.47	82.6	90.2
T. K.	0.21	5.38	53.6	49.7
A. N.	0.071	15.8	100.0	104.0
T. T.	0.17	2.76	24.6	31.6
Mu. T.	0.12	4.59	29.6	33.5
I. S.	0.16	4.30	40.8	59.9
Tt. O.	0.16	8.82	66.9	70.0
T. H.	0.47	11.1	86.6	86.8
Mean	0.20	7.20	55.8	61.7
S. D.	0.11	3.78	22.6	22.8
Pulmonary Fibrosis				
N. A.	0.080	3.90	34.1	49.1
To. M.	0.070	6.20	34.5	50.8
K. T.	0.091	6.26	51.4	59.2
H. I.	0.060	5.90	25.8	27.6
H. T.	0.12	5.20	46.0	48.9
K. O.	0.070	2.40	20.6	45.6
Mean	0.082	4.98	35.4	46.9
S. D.	0.019	1.40	10.6	9.6

Mechanical values in voluntary bradypnea and tachypnea

Six of 8 cases revealed more or less higher value of compliance at tachypnea compared with at bradypnea, while 2 of 8 cases showed almost equal values at each respiratory rate (Fig. 1). As shown in Fig. 2, the values of viscous work of breathing expressed as Gm. M per liter of ventilation held almost constant values from bradypnea to tachypnea, and their range was from 0 to 30 Gm. M./L. As shown in Fig. 3, total work of ventilation was increased both at bradypnea and tachypnea, but the increase in values at tachypnea was almost negligible.

b) Cardiacs

Mechanical values in resting respiration (Table III)

The average value for compliance was 0.096 L./cm. H_2O (S. D. 0.052), and the difference from normal subjects was significant ($p<0.01$). The flow resistances were moderately increased (average, 4.42 cm. H_2O/L./sec., S. D. 2.38). The mean value for viscous work was 37.4 Gm. M./L. (S. D. 16.3), which was apparently lower than the average value of patients with pulmonary emphysema. On the other hand, average value of total work was 53.1 Gm. M./L. (S. D. 19.5), which was in the same order as the value of the patients with emphysema.

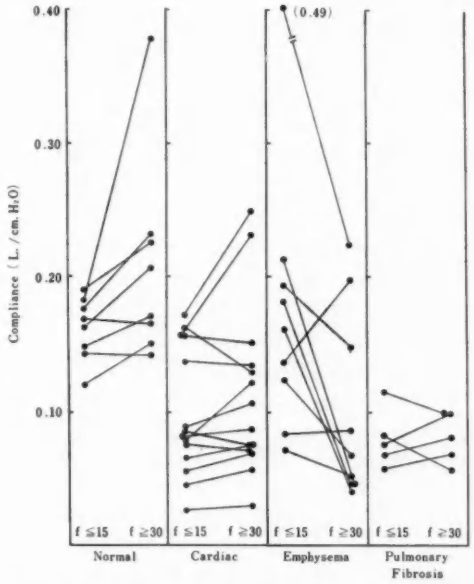

Fig. 1. Effect of respiratory rate on the compliance of normals, cardiacs, patients with emphysema and pulmonary fibrosis.

Mechanical values in voluntary tachypnea and bradypnea.

Three of 14 cases showed significant rise of the values of compliance with increasing respiratory rate from bradypnea to tachypnea, and 2 of

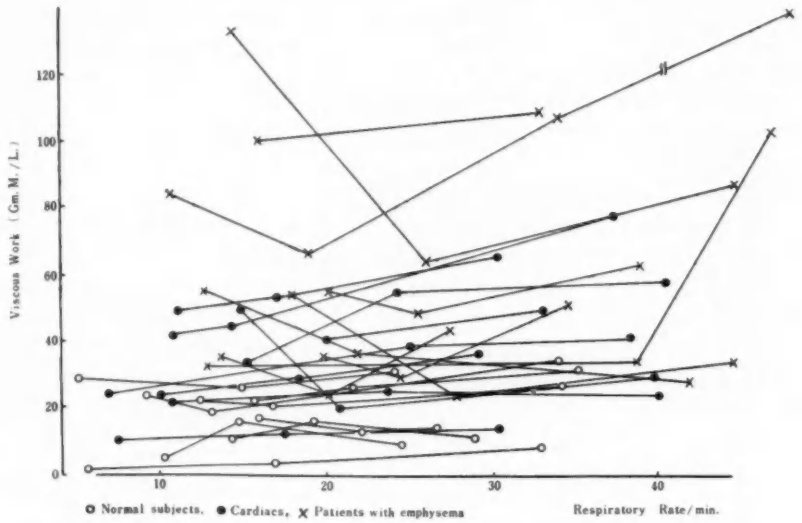

Fig. 2. Effect of respiratory rate on the viscous work per liter of ventilation.

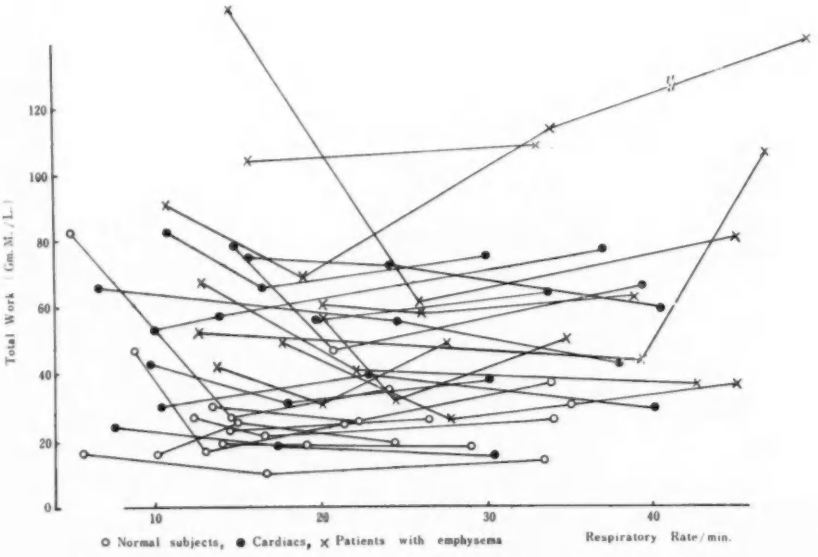

Fig. 3. Effect of respiratory rate on the total work per liter of ventilation.

them had almost normal values of compliance at bradypnea. Other 11 cases revealed no significant change in compliance at various rates of respiration (Fig. 1). Patients who had the value of compliance below 0.15 L./cm. H_2O never had normal value of compliance at any respiratory rate. As shown in Fig. 2, the values of viscous work ranged from 10 Gm. M./L. to 80 Gm. M./L., and, as respiratory rates increased, the work of breathing seemed to increase slightly as a whole, and in the same subject, the differences of the values of works were not so great as emphysema in changing respiratory rate. As shown in Fig. 3, the values of total work were distributed widely from about 20 Gm. M./L. to 80 Gm. M./L., but the influence of respiratory rate on the total works were almost negligible.

Gradient of alveolar N_2 fraction in single breath analysis

Normal values were obtained in all subjects (Table II).

c) Patients with pulmonary emphysema

Mechanical values in resting respiration (Table III)

The values of compliance were widely scattered (range 0.071–0.47), and the average value of compliance was 0.20 L./cm. H_2O (S. D. 0.110), and the difference from normal subjects was not significant ($p>0.05$). The flow resistance showed the highest value in 3 patient groups (average, 7.20 cm. H_2O/L./sec., S. D. 3.78). The mean value of viscous work and total work showed the highest value in 3 patient groups, that is, the mean of viscous work was 55.8 Gm. M./L. (S. D. 22.6), and the average of total work was 61.7 Gm. M./L. (S. D. 22.8).

Mechanical values in voluntary bradypnea and tachypnea

One of 9 cases showed greater compliance at tachypnea than bradypnea, while other 7 patients showed markedly lower values of compliance at tachypnea than at bradypnea. The last one case revealed equal compliance value at each respiratory rate. Then, only 2 cases had the values of compliance below 0.10 at bradypnea, while 6 of 9 cases showed the values of compliance below 0.10 at tachypnea (Fig. 1). As shown in Fig. 2 and Fig. 3, the values of viscous work and total work of breathing were significantly influenced in changing respiratory rate, showing minimal values at respiratory rate of from 20 to 30 per min., namely at near the respiratory rate in resting state.

Gradient of alveolar N_2 fraction

Marked elevation was seen except in one case (Table II).

d) Patients with pulmonary fibrosis

Mechanical values in resting respiration (Table III)

The average value for compliance was 0.082 L./cm. H_2O (S. D. 0.019) and this decrease was significant ($p<0.01$). The flow resistances were moderately increased, and the average of values was 4.98 cm. H_2O/L./sec. (S. D. 1.40). Mechanical works were significantly higher than normals

and lower than the patients with emphysema, the average value of viscous works was 35.4 Gm. M./L. (S. D. 10.6), and the average of total works was 46.9Gm. M./L. (S. D. 9.6).

Mechanical values in voluntary bradypnea and tachypnea

The effect of respiratory rate on the values of compliance was not significant, and the values of compliance were always at abnormally low range at any respiratory rate (Fig. 1).

Gradient of alveolar N_2 fraction

The values distributed from normal range to moderate elevation (Table II).

Discussion

a) Cardiacs

The values of compliance obtained in cardiacs were in the same order of the values of previous reports[5),6),8),12),13),16)-18)] and significantly lower than those obtained in normal subjects in the present report, though 3 cases had normal value of compliance. Moreover, the values of compliance were almost invariable except in 2 cases who had normal value of compliance, when respiratory rate was actively changed from resting state. On the other hand, the gradient of alveolar N_2 fraction, which was measured to evaluate the uneven ventilation in the tracheobronchial tree, was completely in normal range. The facts show the evenness of ventilation, or equal time constants of each alveolar unit, notwithstanding the existence of uneven ventilation.[27),30)] In the latter case, the decrease in compliance of each alveolar unit is accompanied by the increase in resistance leading to the respective alveoli. The steadiness of compliance in changing respiratory rate will occur in the same manner in these 2 different states of ventilation. In cardiacs, the flow resistances were moderately increased. It is difficult to ascribe these increased resistance to increased tissue resistance only.[5)] Then increase in airway resistances will certainly attend the uneven distribution in different airways. Therefore, existence of uneven ventilation with equal time constant in each unit is most probable in cardiacs.

Almost constant values of total work per L. of ventilation in various respiratory rates and slight increase in viscous work per L. of ventilation with the increase in respiratory rate, suggest that the mechanical efficiency of ventilation is relatively invariable throughout wide range of respiratory rate. The relation between respiratory rate and viscous or total work was very similar to that in normals, and differed significantly from that in emphysema.

b) Patients with pulmonary emphysema

The results obtained in the patients with chronic pulmonary emphysema generally agreed with those of previous reports,[1),2),4),9),11),13),15),23),24)] except that compliance did not show significant reduction as a whole in the resting state. As the effective compliance depends on respiratory frequency markedly, decreased compliance in previous reports[9),20),23),24)] may be due to the measurement in tachypneic state.

The viscous and total work done on the lungs varied significantly when respiratory rate was changed, giving minimal value at near the resting frequency, namely from 20 to 30. These results made a sharp contrast with normals and cardiacs. For the total work, the increase at bradypnea may be explained by the attending small increase in tidal volume, making the elastic work per L. greater. However, for the viscous work, similar explanation could not be applied. In the lungs of patients with emphysema, in which elasticity of alveoli are lost and marked obstruction of small bronchi and bronchioles exist, the hysteresis phenomenon should not be regarded as a negligible factor. The marked variation of viscous and total work with respiratory rate observed in emphysema would be explained at least partly on the base of hysteresis phenomenon. In addition, we pointed out in the pneumotachogram of patients with emphysema, that prolongation of expiratory phase in emphysema and asthma may be interpreted as an adaptation phenomenon, making the high resistant expiration slower and the low resistant inspiration more rapid.[25)] The existence of minimal work at resting rate of respiration in patients with emphysema would be also risen from the maximal expiratory time to inspiratory time ratio at resting state. However, the problem which one of these 2 factors is more important in the genesis of minimal work at resting state is

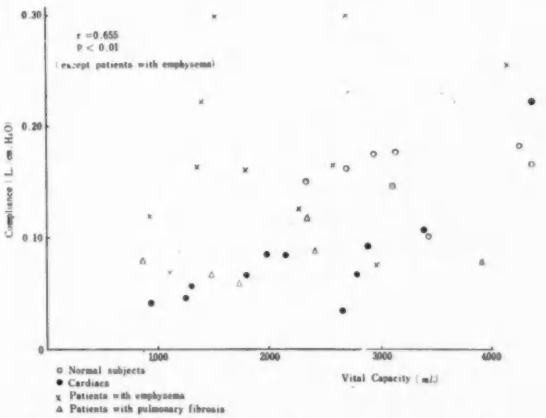

Fig. 4. Relation between compliance and vital capacity.

not yet clear in this study.

c) Physical characteristics of cardiacs and patients with emphysema—comparison of 2 ventilatory disturbance

The results obtained for the mechanical properties of the lungs indicated that there existed marked difference between patients with emphysema and cardiacs or pulmonary fibrosis. In addition, the relationships between mechanical values and spirometric volumes show that the pattern of ventilatory disturbance of chronic pulmonary emphysema was quite different from that of cardiacs or patients with pulmonary fibrosis. In the relation between compliance and vital capacity, good correlation was seen for the normals, cardiacs and patients with pulmonary fibrosis ($r=0.655$, $p<0.01$), while the correlation was not significant for the whole subjects including emphysema (Fig. 4). Between compliance and MMF, similar exceptional behavior of emphysema was seen (Fig. 5). In the rela-

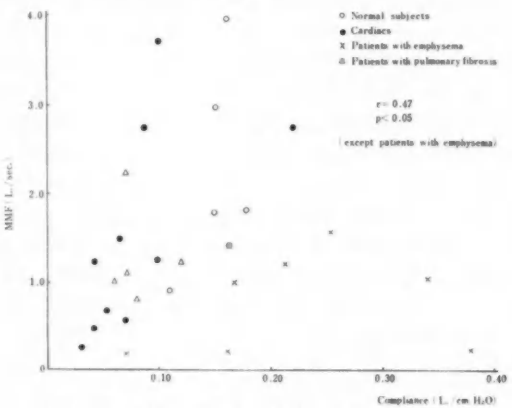

Fig. 5. Relation between MMF and compliance.

tion between flow resistance and the gradient of alveolar N_2 fraction by single breath analysis (Fig. 6), the normal subjects showed low N_2 gradient and low flow resistance, but patients with emphysema and pulmonary fibrosis showed high N_2 gradient and high flow resistance. In cardiacs, despite the moderate increase in flow resistance, the gradients of alveolar N_2 fraction were in normal range.

In summarizing the present results, the essential characteristics of pulmonary emphysema are 1) dependency of elasticity of lung on respiratory rate, 2) significant uneven distribution, 3) high flow resistance and 4) significant rise in ventilatory work in bradypnea and tachypnea. While in cardiacs, the essential characteristics are 1) rigidity of the lung, 2) not significant uneven distribution of ventilation, 3) moderately increased

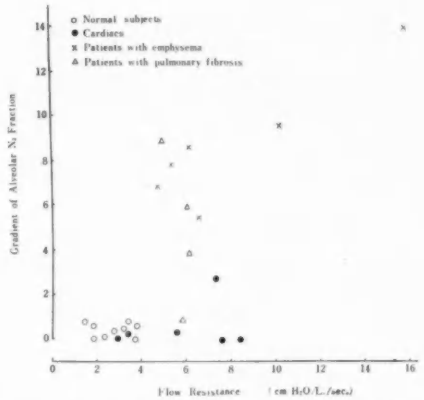

Fig. 6. Relation between flow resistance and gradient of alveolar N_2 fraction.

flow resistance and 4) relatively constant values of ventilatory work. The mechanical behavior of the patients with pulmonary fibrosis would be standing between cardiacs and the patients with emphysema.

Summary

Effective compliance, average flow resistance and mechanical work were measured by intraesophageal pressure technic in following subjects: 14 normals, 14 cardiacs, 11 emphysematous and 6 patients with pulmonary fibrosis. The influence of respiratory rate on these values and gradient of alveolar N_2 fraction by single breath analysis were also studied, and results were as follows.

(1) In cardiacs, compliances were significantly decreased, and flow resistances and viscous work were moderately increased, while total work was markedly increased. In changing respiratory rate, compliances and mechanical works remained stable. Gradients of alveolar N_2 fraction were in normal range.

(2) In emphysematous patients, compliances were widely scattered and the difference from normal subjects was not significant, though markedly decreased in tachypnea. Flow resistances and mechanical works were markedly elevated. In changing respiratory rate from resting state, marked elevation of mechanical works was noted. Gradients of alveolar N_2 fraction were markedly elevated.

(3) In patients with pulmonary fibrosis, decrease in resting compliance and moderately increased flow resistance and mechanical works

were noted. Effect of changing respiratory rate on mechanical values was not marked. Gradients of alveolar N_2 fraction were moderately elevated.

(4) These results indicated that the mechanical behavior in cardiacs and patients with pulmonary fibrosis made sharp contrast with that in the patients with emphysema.

Acknowledgement

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Experimental Studies on Cerebral Hemorrhage

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With the hope to clarify the relationship between vasospasm and cerebral vascular lesion, the cerebral arteries of dogs were occluded. By occluding 2 or 3 cerebral arteries, the incidence of cerebral hemorrhage becomes remarkably higher than by the occlusion of the middle cerebral artery alone. The primary cause of hemorrhage is considered to consist of arterial engorgement by sudden occlusion of pial arteries with subsequent venous stasis, and the angionecrosis is emphasized to be due to the result of blood invasion into the vessel wall from the lumen. It is concluded that if vasospasm has something to do etiologically with cerebral hemorrhage, its sites of origin may be in pial arteries.

ALTHOUGH numerous hypotheses have been reported, the pathogenesis of cerebral hemorrhage is not fully understood. Among those concepts related to functional mechanism of cerebral hemorrhage, the role of vasospasm has been particularly a matter of controversy. For a long time, cerebral vasospasm has been thought to be a cause of transient or recurrent strokes in patients suffering from hypertension or arteriosclerosis (Peabody,[1] Pal,[2] Kauffman[3] and Westphal and Bär[4]). Although cerebral vasospasm itself has been ascertained by some researchers, its significance for cerebral vascular lesions is not at all conclusive. Those who deny its significance insist on the following facts: the innervation of cerebral vessels is not very extensive (Forbes et al.[5]); smaller cerebral arteries are partially lacking in muscular layer (Barker[6]); there is a close similarlity clinically and pathologically between the attacks in hypertension and in cerebral embolism (Pickering[7]); and experimentally the diameter of the pial vessels cannot be changed by external stimulation except changes following those in systemic blood pressure (Rothenberg and Corday[8]). On the other hand, Pool, Jacobson and Fletcher[9] demonstrated that the larger cerebral arteries in man were capable of vasoconstriction clinically and experimentally. Pool[10] postulates that cerebral vasospasm may lead to permanent neurologic deficit or fatal

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cerebral edema. Recently one of the authors Ono,[11] reported a microscopic study on cerebral vasoconstriction and found the remarkable descent of stimulus-threshold in pial vessels of rabbits previously subjected to renal or carotid artery constriction. The degree of the vasospasm was extremely wide and severe and the duration of spasm was also long. However, such topical electric stimulus had no effect on blood pressure.

These findings led us to make further experiments to ascertain what results might be obtained in case of more prolonged vasospasm. With the hope to clarify the relationship between vasospasm and cerebral vascular lesion, we occluded cerebral arteries of the dog permanently and made the observations.

Materials and Methods

Eighty female dogs each weighing about 10 Kg. were used for the study. Dogs were anesthetized with the intravenous injection of pentobarbital calcium (Labonal) solution. This required 0.3 to 0.5 Gm. of the anesthetic agent at the beginning of the operation and if necessary additional injections were repeated during the operation. Under aseptic technic, the scalp of the dog was incised vertically on one side and exposed zygomatic arc was resected. After partial resection of mandible, the heavy temporal muscle was split vertically and retracted by the wound-extender. Near the base of the skull the temporal bone was resected as widely as possible. After opening dura, the objective cerebral arteries were occluded with silver clips (0.5 cm. × 0.08 cm.). These include anterior, middle and posterior cerebral, basilar and anterior superior cerebellar arteries. In the majority of cases 2 arteries among these arteries, e.g. anterior and middle cerebral or middle cerebral and basilar arteries, were occluded together. In 4 dogs the anterior spinal artery was occluded after laminectomy of the third and fourth cervical vertebrae. After occlusion of arteries, muscle and skin were sutured and penicillin was injected.

The period of clinical observation after the operation varied case by case from 1 to 177 days. During coma, the dog was nourished by glucose injection. Autopsy was performed in all cases immediately after death or sacrifice by intravenous injection of anesthetic agent and potassium chloride.

The brains, after fixation in formalin, were sectioned coronally at about 5 mm. intervals and examined macroscopically about the development of hemorrhage or softening and the representative sections were taken for a histological examination including serial sections. All sections were stained with hematoxylin and eosin, Weigert elastica stain with van Gieson, Azan stain and benzidine-method.

Results

1. Uncombined occlusion of the middle cerebral artery (Table I)

In a total of 27 dogs examined, one died next day of the operation,

Table I. Results of Occlusion of the Middle Cerebral Artery Alone

Dog number	Side of operation	Postoperative day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Period of existence (days)	Gross appearance of brain
1	Right	+	−	−	−	−	−	△														7	No abnormality
2	〃	‡	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	△			18	〃
3	〃	‡	±	±	−	−	−	−	−	−	−	−	−	−	−	−	×					16	〃
4	〃	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	25△	Minimal hemorrhage
5	〃	‡	‖	‖	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	35△	Mild hemorrhage
6	〃	‡*	‖	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	35△	〃
8	Left	‡	‡	×																		3	No abnormality
9	〃	+	±	±	±	±	±	±	△													8	〃
10	〃	‖	−	−	−	−	−	△														7	Mild hemorrhages
11	〃	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	39△	No abnormality
12	Right	‖	+	+	±	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	177△	〃 , operated twice, see Table II.
13	Left	‡	‖	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	71△	No abnormality
14	〃	‡	‖	+	+	+	+	+	×													8	〃
15	〃	+	±	±	−	−	−	−	−	−	‡	×										11	Minimal hemorrhage
16	Right	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	×				17	No abnormality
17	〃	+	+	+	±	±	±	±	±	±	±	±	×									12	〃
18	Left	+	−	−	−	−	−	−	−	−	−	△										11	〃
19	Right	‖	‡	×																		3	〃
20	Left	‡*	‡	‡	‖	‖	+	△														7	〃
21	〃	‖	‖	‖	‖	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	92×	〃 , operated twice, see Table II.
24	Right	×																				1	〃
25	Left	‖	‖	‖	+	+	+	±	−	−	−	−	−	−	−	−	−	−	−	−	−	107△	〃
26	〃	‖	‖	‖	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	37△	〃
27	Right	‡*	+	+	+	+	+	+	+	+	+	±	±	±	±	±	±	±	±	±	±	60△	Extensive softening
29	〃	‡	‖	‖	‖	‖	‖	‖	‖	‖	‖	‖	‖	×								13	Moderate softening
32	〃	‡	‡	‡	‖	‖	+	+	+	+	+	+	+	+	+	‡	‡	×				17	Mild hemorrhagic softening
48	〃	‡	‖	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	88△	No abnormality

‡ coma; ‖ severe hemiplegia, standing impossible; ‖ moderate hemiplegia, standing possible; + mild hemiplegia, walking possible; ± hemiparesis; − normal; * convulsion; × death; △ sacrifice

Table II. Results of Combined Occlusion of the Anterior and Middle Cerebral Arteries

Dog number	Side of operation	Postoperative day																				Period of existence (days)	Gross appearance of brain
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
12	Left	⫴	⫲	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	73△	No abnormality
21	Right	⫴	×																			2	Mild hemorrhage
28	Left	⫴	⫴	⫲	⫲	⫴	×															6	Massive hemorrhage
33	〃	⫴*	⫴	×																		3	〃
34	Right	⫴	×																			2	〃
40	〃	⫲	⫲	⫲	⫲	⫲	×															6	Mild hemorrhage
42	〃	⫲	⫲	⫲	⫲	⫲	×															6	〃
43	〃	⫴*	⫴*	⫲	⫲	⫲	⫲	⫲	+	±	±	−	−	−	△							14	Minimal hemorrhage
44	Left	⫲	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	94△	No abnormality
45	Right	⫲	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	91×	〃 , operated twice, See Tab. III
46	Left	×																				1	Mild hemorrhage
47	〃	⫴	⫲	+	+	+	+	+	+	+	+	+	⫲	×								13	Minimal hemorrhage
49	Right	⫴	⫲	⫲	⫲	⫲	⫲	△														7	〃
50	Left	⫴	⫲	⫲	⫲	⫲	⫲	⫲	⫲	⫲	⫲	⫲	⫲	⫲	+	+	+	+	+	+	+	81△	État lacunaire

2 died 3 days after the operation and 7 died within 8 to 17 days after the operation. Minimal hemorrhage was found macroscopically in 2 of these dogs (No. 4 and 15) and moderate pale and hemorrhagic softening in one case respectively (No. 29 and 32). All other 17 dogs were sacrificed within 7 to 177 days after the operation. Macroscopic lesions were found in 4 dogs including mild hemorrhage in 2 (No. 6 and 10) and moderate and extensive softening in one respectively (No. 5 and 27).

Clinically, these dogs recovered quickly from severe hemiplegia in most of the cases. Three days after the operation, severe hemiplegia was observed in 2 and moderate hemiplegia in 5 dogs. Seven days after the operation, only one dog (No. 29) showed moderate hemiplegia.

2. Occlusion of the other arteries

a. Combined occlusion of the anterior and middle cerebral arteries (Table II)

Fig. 1. Massive hemorrhage accompanied by ventricular hemorrhage (No. 34).

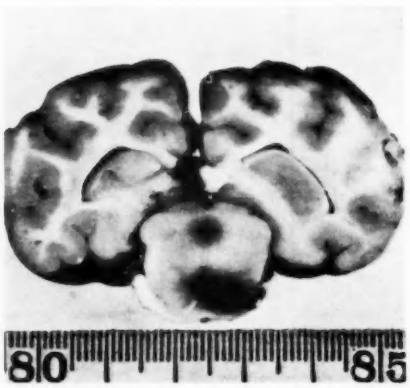

Fig. 2. Massive hemorrhage in the pons by occlusion of the middle cerebral and basilar arteries (No. 77). This case is not included in tables.

Out of 14 dogs in total, 4 died one or 2 days after the operation without regaining consciousness, 3 died 7 days after the operation in severe hemiplegia and one died 13 days after the operation. Remaining 6 were sacrificed within 7 to 94 days after the operation. Macroscopic lesions were observed in 11 out of 14 cases, including massive hemorrhage in 3 (No. 28, 33 and 34), mild hemorrhage in 4 (No. 21, 40, 42 and 46), minimal hemorrhage in 3 dogs (No. 43, 47 and 49) and *état* lacunaire in one case (No. 50).

b. Combined occlusion of the middle and posterior cerebral arteries (Table III)

Table III. Results of Occlusion of the Other Cerebral, Cerebellar and Spinal Arteries

Dog number	Side of operation	Occluded arteries	Postoperative day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Period of existence (days)	Gross appearance of brain
35	Right	M.P.	×																				1	Massive hemorrhage
36	〃	M.P.	⫼*	⫼*	⫼	⫽	⫽	⫽	⫽	+	+	+	+	+	+	+	+	+	+	+	+	+	64×	No abnormality
37	〃	M.P.	⫼*	⫼*	+	+	+	+	⫽*	⫽*	⫽*	×											10	Mild hemorrhage
39	〃	M.P.	⫼	⫽	⫽	⫽	⫽	⫽	⫽	⫽	⫽	⫽	⫽	⫽	⫽	⫽	⫽	⫽	×				17	Large softening
36	Left	A.M.P.	⫼	×																			2	Mild hemorrhage
51	Right	A.M.P.	⫼	⫼	×																		3	〃
52	Left	A.M.P.	⫼	⫼	⫽	⫽	⫽	⫽	⫽	⫽	×												9	Extensive hemorrhagic softening
53	Right	A.M.P.	⫼	⫼	⫼	⫼	×																5	Mild hemorrhage
57		B	×																				1	Massive hemorrhage
59		B	+	+	−	−	−	−	−	−	−	−	−	−	−	−	△						15	No abnormality
60		B	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	34△	〃
65		B	⫽	+	−	−	−	−	−	−	⫽	⫽	△										11	Internal hydrocephalus
66		B	⫽	+	−	−	−	−	−	−	−	−	△										11	Minimal hemorrhage
56	Right	M.B.	⫽	×																			2	Scattered hemorrhage
54	R.L.	P.B.	×																				1	Mild hemorrhage
55	Right	P.B.	×																				1	〃
25	〃	S.B.	⫼	⫽	⫼	±	−	−	−	−	−	−	−	−	−	−	−	−	−	△			18	〃
61	Left	S.B.	⫽	⫽	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	31△	Minimal hemorrhage
62	Right	S.B.	⫽	×																			2	No abnormality
63	〃	S.B.	⫽	⫽	⫽	⫽	⫽	⫼	⫽	⫽	×												9	Minimal hemorrhage
45		Spi.	×																				1	Massive hemorrhage
67		Spi.	×																				1	〃
69		Spi.	⫼	⫽	⫼	⫽	⫽	⫽	△														7	Large softening
70		Spi.	×																				1	Massive hemorrhage

A. anterior cerebral artery; M. middle cerebral artery; P. posterior cerebral artery; B. basilar artery; S. superior anterior cerebellar artery; Spi. anterior spinal artery

Out of 4 dogs in total, one died next day of the operation (No. 35), showing massive hemorrhage. One died 10 days after the operation (No. 37), showing mild hemorrhage and another died 17 days after the operation, showing large softening (No. 39).

c. Combined occlusion of the anterior, middle and posterior cerebral arteries (Table III)

Four dogs were used in this group and 3 died in coma within 5 days following the operation and one died 9 days after the operation in moderate hemiplegia. Mild hemorrhage was observed in 3 cases (No. 36, 51 and 53) and extensive hemorrhagic softening in one case (No. 52).

d. Uncombined occlusion of the basilar artery (Table III)

In a total of 5 dogs, only one (No. 57) died in coma next day of the operation, showing massive hemorrhage in pons. Other 4 animals recovered from hemiplegia completely within 2 to 3 days after the operation, but one became subcomatose again and the autopsy revealed an internal hydrocephalus (No. 65), and small hemorrhage in pons was observed in another dog by sacrifice (No. 66).

e. Combined occlusion of the basilar and other arteries (Table III)

The one in which the basilar and middle cerebral arteries were occluded died in coma 2 days after the operation and scattered hemorrhages were observed in both cerebral hemispheres, midbrain, pons and cerebellum (No. 56).

Two dogs (No. 54 and 55) in which the basilar and posterior cerebral arteries were occluded died in coma following day of the operation and mild hemorrhage was observed in occipital lobe and in pons respectively.

Out of 4 animals in which the basilar and the superior anterior cerebellar arteries were occluded, 3 were found to have mild hemorrhage in pons (No. 25), minimal hemorrhages in occipital lobe and cerebellum (No. 61) and minimal hemorrhage in pons (No. 63) respectively. Only one case (No. 62) showed no macroscopic lesion in spite of dying 2 days after the operation.

f. Uncombined occlusion of the anterior spinal artery (Table III)

Three out of 4 dogs (No. 45, 67 and 70) died following day of the operation showing spindle-shaped hemorrhage surrounding the central canal. The other case (No. 69) was sacrificed 7 days after the operation and showed softening of about 2 cm. in diameter.

3. Uncombined occlusion of larger pial veins (Table IV)

In a total of 10 dogs used in this group, 2 died 2 days after the operation, showing no macroscopic lesion. Remaining 8 were sacrificed within 4 to 28 days after the operation when no symptom was observed clinically. Only 2 dogs (No. 106 and 116) showed minimal hemorrhage in the cortex near the site of the occlusion.

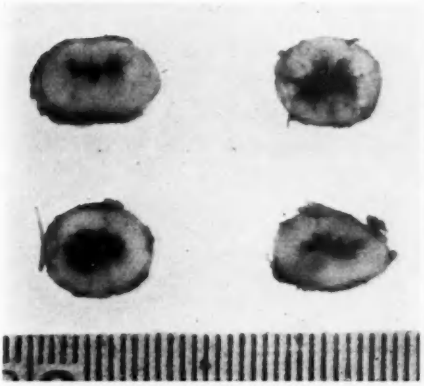

Fig. 3. Massive hemorrhage in the spinal cord (No. 67).

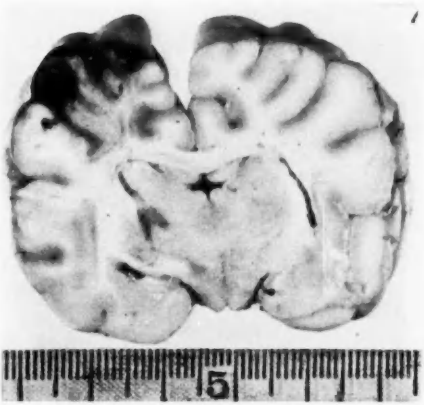

Fig. 4. Small hemorrhages in the cortex (No. 116).

Table IV. Results of Occlusion of Larger Pial Veins

Dog number	Occluded sinus and veins	Postoperative day 1	2	3	4	5	6	7	Period of existence (days)	Gross appearance of brain
106	Sinus petrosus sup.	++	−	−	−	−	−	−	10△	Minimal hemorrhage
107	V. jugularis int.	+	+	±	△				4	No abnormality
108	Sinus transversus	++	+	±	−	−	−	−	10△	〃
109	Vv. cereb. dorsales	−	−	−	−	−	−	−	28△	〃
110	Vv. cereb. lat. et vent.	+	−	−	−	−	−	△	7	〃
111	Vv. cereb. dorsales	++	×						2	〃
112	Vv. cereb. dors. et sinus sagittalis sup.	++	−	−	−	−	−	−	18△	〃
113	〃	?	×						2	〃
114	〃	++	+	+	+	±	±	−	11△	〃
116	〃	++	±	−	△				4	Minimal hemorrhage

4. Histological examination

In the histological examination of the areas of hemorrhage and their surroundings, we found angionecrosis, vascular dilatation, hyalinosis and crack formation of the vessel walls, thrombus formation in the lumen of the vessel, plasma escape from the lumen of the vessel and multi-development of minimal aneurysms closely similar to the pathology of cerebral hemorrhage in man. Observations on slides prepared with benzidine also showed a marked dilatation of the vessels. Further, we found aneurysms involving perforating deep branches of the middle cerebral artery in one case.

Although many modes of reaction of smaller intracerebral vessels may be found in cerebral hemorrhage, the most marked alterations are as follow; a marked increase in the dimensions of the vessel and so-called angionecrosis. We consider the former as venous and the latter as arterial in origin.

In complete angionecrosis, the vessel wall appears to have no structure at glance, but a thorough examination of many preparations revealed numerous transitional stages. In Fig. 5, inner and outer layers of the

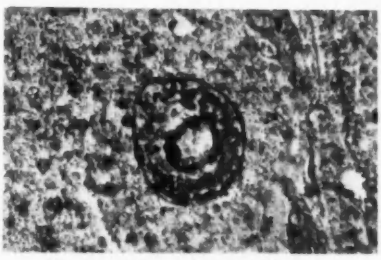

Fig. 5. Azan stain, ×180.

Fig. 6. Azan stain, ×180.

vessel wall are splitted and are filled with blood cells as well as in the lumen. In Fig. 6, the lumen is full of blood plasma with some leukocytes, while in the wall occasional blood cells can be seen. Of the right arteriole of Fig. 7, the media and the internal elastic layer remain preserved, but the adventitia is splitted resulting in crack formation of the wall with invasion of some blood cells. The right and left arterioles are connected by connective tissue layer. Although the media of the latter is still seen, the wall is filled with blood cells resulting in a rupture of the wall. In Fig. 8 blood cells in the wall as well as the lumen cannot be discriminated individually, showing the appearance as if they yielded to coagulative necrosis. The mild hemorrhage outside of the vessel shows similar appearance. Fig. 9 shows the so-called "ring hemorrhage", inside of which the complete necrosis of the wall is observed but in the lumen of this vessel only a few leukocytes in blood plasma are present. Between the "ring hemorrhage" and the degenerated vessel wall, there is seen the brain parenchyma. In Fig. 10, red blood cells are present in the middle of the vessel lumen enclosed by white blood cells, outside of which one can see a trace of degenerated wall.

From these findings we supposed that the angionecrosis may be due to the invasion of blood into the vessel wall from the lumen resulting in so to speak coagulative necrosis of the vessel wall. Furthermore, from the finding of the invasion of blood into the vessel wall through a rent of the intima, we supposed that the dissecting hemorrhage of the vessel wall

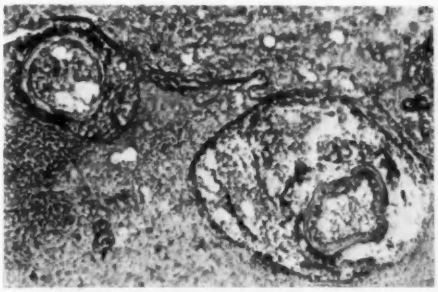

Fig. 7. Azan stain, ×180.

Fig. 8. Azan stain, ×180.

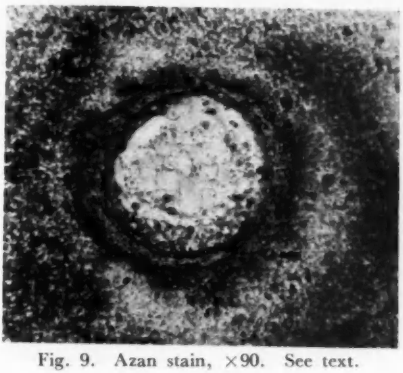

Fig. 9. Azan stain, ×90. See text.

Fig. 10. Azan stain, ×180.

may be the primary cause of the angionecrosis. However, the possibility that the invasion of blood into the vessel wall may originate from the change in permeability of the vessel wall cannot be denied.

Contrary to the findings in the artery, smaller intracerebral veins show striking dilatation, resulting in very thin vessel wall composed of only a few strands of collagenous tissue (Fig. 11). Fig. 12 shows globular dilatations of smaller veins. Similar globular dilatations of smaller veins are seen at the lower and upper part of Fig. 13. Around the irregular shaped hemorrhages seen at the middle and right lower part of Fig. 13, one can see a very thin strand of connective tissue.

Occasionally, in microscopic examination, we may see the proliferation of capillaries in the brain in which no macroscopic lesion is noted (Fig. 14). It is yet unknown whether this proliferation of capillaries is the precursor of hemorrhage or functional reaction to the circulatory disturbance. In the histological examination of the brain subjected to the occlusion of larger pial veins, there can be seen capillary hemorrhages due to permeation and a marked increase in thrombus formation in the

lumen of smaller vein without any changes of vessel wall as seen in massive hemorrhage (Fig. 15).

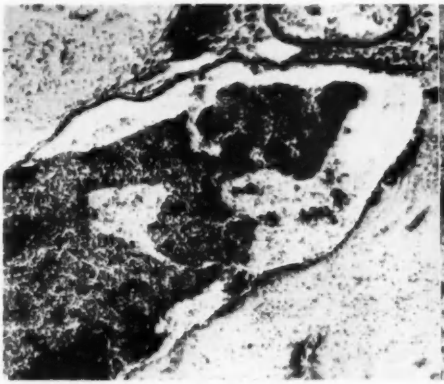

Fig. 11. Azan stain, ×90.

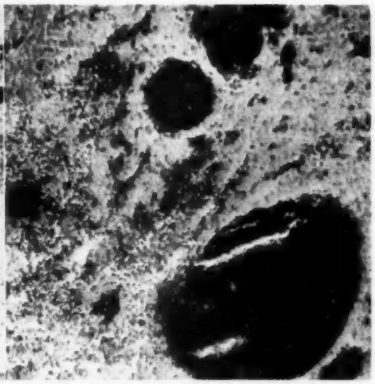

Fig. 12. Azan stain, ×90.

Fig. 13. Azan stain, ×27.

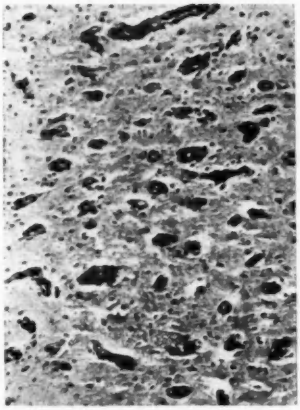

Fig. 14. Hematoxylin and eosin stain, ×27.

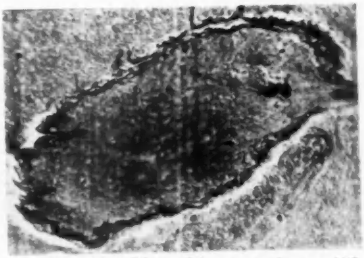

Fig. 15. Van Gieson stain, ×180. See text.

Discussion

Westphal, who attaches great importance to angiospasm in the pathogenesis of apoplexy, maintains an opinion that in some localized area of the brain there takes place an ischemia of the parenchyma due to vasospasm resulting in an acute necrosis of the parenchyma and vessels; and that once the spasm is over the circulation is resumed in the altered vessels with subsequent hemorrhage brought on by diapedesis and vascular injury.

Fisher-Wasels[12] suggests that the vasospasm is determined by an abnormal stimulus, but he adds that the spasm is in some cases followed by the circulatory stasis and that only in consequence of this stasis do alterations of cerebral metabolism take place resulting in the production of vaso-dilatory and other substances capable of modifying the permeability of the vessel walls, even of producing angionecrosis with subsequent hemorrhages.

On the other hand, we succeeded in producing cerebral hemorrhage by occluding 2 or 3 pial arteries. As Pool demonstrated experimentally and clinically, larger cerebral arteries in man are capable of vasoconstriction. Therefore, it is very possible that a sudden occlusion of pial arteries can occur not only by embolus or thrombus but also by vasospasm. If the spasm has pathogenetically anything to do with cerebral hemorrhage, it may be assumed that the site of the angiospasm is in pial arteries and not in smaller intracerebral arteries as supposed by Westphal. Furthermore, in our experiments, occlusion of the middle cerebral artery alone was unsuccessful in producing massive hemorrhage, but the occlusion of 2 or more pial arteries was successful in producing hemorrhage in most cases. This suggests that the anastomoses of pial arteries are very important for the regulation of the cerebral circulation.

The classic conception that the arterial system of the encephalon is absolutely terminal has been questioned by many researchers (Pfeifer,[13] Spatz[14] and Motosato[15]). Recently, Ecken[16] has rediscovered a great number of anastomotic channels between leptomeningeal arteries of the brain. Although he found no actual anatomical anastomosis of any significant degree between the perforating deep branches of cerebral arteries, there may be, we suppose, at least functional anastomotic networks between these deep branches. By these anastomoses between the leptomeningeal and the deep perforating arteries, occlusion of the middle cerebral artery alone does not result in severe circulatory stasis except some ischemic softening or mild hemorrhage in several cases. On the contrary, in cases of occlusion of 2 or more pial arteries, circulatory stasis takes place in those areas supplied by proximal branches of the occluded arteries including

the deep perforating arteries. In such a stasis we find both arterial engorgement and venous stasis. Thus, successive alterations of the vessel walls may take place as suggested by Fisher-Wasels.

On the other hand, from the histological view point the direct cause of cerebral hemorrhage has been studied by many researchers. Although it is the most convincing opinion that the apoplectic cerebral hemorrhage is caused by the rupture of arteries due to the acute angionecrosis (Stämmler,[17] Beitzke,[18] Matsuoka[19] and Ooneda[20]), Scheinker[21] believes that the rupture of altered veins may produce the same picture. From the histochemical study of Ooneda, the angionecrosis is considered to be a fibrinoid degeneration of cerebral vessels. He also points out that as the result of an abnormally increased vascular permeability, blood plasma and fibrin permeate through the vascular wall to be deposited in the wall as fibrinoid substance.

In the histological study of experimentally produced cerebral hemorrhages, we found that both angionecrosis and venous dilation might be the cause of the hemorrhages. Moreover, we obtained findings that the angionecrosis is considered to be the result of blood invasion into the vessel wall by dissecting the intima. According to our points of view, the arterial walls are strong enough to tolerate the elevated intravascular pressure due to circulatory stasis and may not show significant dilatation, while the venous walls oppose this pressure simply by dilatation. But once the pressure exceeds the limit, by dissection of intima or abnormally increased permeability, the blood invades into the wall of the artery resulting in, so to speak, coagulative necrosis and the extremely dilated vein shows itself as a ball hemorrhage which is the unit of cerebral hemorrhage according to Scheinker.

With a hope to determine which is more pertinent as a direct cause of hemorrhage, rupture of arteries or that of veins in the brain, we occluded larger pial veins. This experiment, however, was unsuccessful in producing massive cerebral hemorrhage. Also in the histological examination of the surrounding areas of minimal hemorrhage, we found as a dinstinct finding intracranial venous dilatations with thrombus formation instead of the angionecrosis or ball hemorrhage. Accordingly, we consider that ruptures of both arteries and veins are important as a direct cause of cerebral hemorrhage though the primary cause of hemorrhage may consist of arterial engorgement resulting in secondary venous stasis.

Globus[22] reported examples of a massive cerebral hemorrhage occurring in preexisting softened area. In his experimental study, he was able to produce a larger area of the softening following the occlusion of the middle cerebral artery in the brain of the dog injected neosynephrin intravenously and successfully produced a massive cerebral hemorrhage.

Hain, Westhaysen and Swank[23] described hemorrhagic cerebral infarction by occluding the middle cerebral artery in dogs. But they were able to produce hemorrhagic infarcts only when the site of occlusion was within the zone of origin of the perforating deep branches, whereas occlusion of the artery distal to the zone of origin of the perforating branches resulted in only a pale infarct. Moreover, in monkeys, arterial occlusion resulted in a pale infarct regardless of the site of occlusion of the artery. They postulated that the different response in the 2 animals is due to a difference of collateral circulation distal to the point of the occlusion.

It is not conclusive that the difference between the results obtained by Globus and Hain et al. are primarily due to the difference in the site of the occlusion. In our experiments, the occlusion of the middle cerebral artery alone resulted in a relatively large pale infarct in some cases but not massive hemorrhage. In these experiments we selected the site of occlusion in immediately distal part to the zone of origin of the perforating deep branches.

In human pathology, the hemorrhagic infarction and the incipient brain metastasis of the lung cancer are almost always found in cortex or subcortex, whereas the localization of cerebral hemorrhage is usually paralenticular. This fact led us to suspect that in cerebral hemorrhage something might happen in the distal part of the zone at which the perforating deep branches originate.

According to the results of Glous, Hain et al. and ours, there is no evidence that the hemorrhage took place in the previously softened area. Circulatory disturbance by occlusion of the artery may rather be more important for the development of hemorrhage. Fazio[24] described that the hemorrhagic infarct is not brought about by purely passive and mechanical factors but by active phenomena of vaso-dilation with consequent stasis followed by diapedesis, but he also mentioned that it is less evident whether the pathogenetic mechanism of such a phenomenon is due to vaso-vascular reflexes from some remote stimulus or whether it is due to a local action of vaso-dilatory substances produced by a previously disturbed circulation.

By experimental study of cerebral embolism,[25] we confirmed Fazio's hypothesis that in hemorrhagic softening, the area supplied by the same arterial branch may be in part ischemic and partly in hyperemic stasis. It may be postulated then that a sudden occlusion of an arterial branch by embolus may bring about inevitably stasis in adjacent area just as postulated in massive hemorrhage by the occlusion of pial arteries.

Summary

With a hope to clarify the relationship between vasospasm and cerebral hemorrhage, we made occlusion of cerebral arteries of the dogs. After clinical observation of period varying from several days to a few months, dogs were sacrificed and macroscopic and histologic examinations were performed.

(1) Occlusion of the middle cerebral artery alone resulted in mild hemorrhage in 2, large softening in one and moderate softening in 3 out of 27 dogs studied. Clinically, dogs recovered from severe hemiplegia quickly in most of the cases.

(2) Combined occlusion of the anterior and middle cerebral arteries resulted in massive hemorrhage in 3, mild hemorrhage in 4, minimal hemorrhage in 3 and *état* lacunaire in one among a total of 14 dogs in this group. Clinically dogs died in coma or severe hemiplegia in almost all instances.

(3) Combined occlusion of 2 or more arteries including middle and posterior cerebral, basilar, superior anterior cerebellar and anterior spinal arteries resulted in massive hemorrhage in 5, mild hemorrhage in 7, minimal hemorrhage in 3, large softening in 3 and internal hydrocephalus in 1 out of 23 dogs.

(4) These experiments showed that by occluding 2 or more cerebral arteries, the incidence of cerebral hemorrhage becomes remarkably higher than by occlusion of the middle cerebral artery alone. This may indicate the importance of circulatory regulation by anastomotic channels. But the areas supplied by the anterior spinal artery showed a high incidence of vascular lesion due to its occlusion alone.

(5) In the histological examination of the areas of hemorrhage or their surroundings, there can be seen the same changes of the vessel walls as seen in the human pathology of cerebral hemorrhage.

(6) On the other hand, by the occlusion of larger pial veins we were unable to produce a larger hemorrhage. Also in the histological examination of the surrounding areas of minimal hemorrhage, we found a marked increase in thrombus formation in the lumen of smaller veins instead of the changes in vessel walls as seen in massive hemorrhage by arterial occlusion.

(7) Although both angionecrosis and venous dilatation may be the direct cause of hemorrhage, we consider an arterial engorgement by sudden occlusion of pial arteries with subsequent venous stasis may be more pertinent. Furthermore, it is postulated that the angionecrosis may be the result of blood invasion into the vessel wall from vessel lumen either

by the dissection of the intima or by the change in permeability of the vascular wall.

(8) On the bases of above mentioned data, it is concluded that if vasospasm play a role in cerebral hemorrhage, it may well be in pial arteries resulting in circulatory stasis in the area supplied by their proximal branches.

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Antiatherosclerotic and Anticoagulant Activity of Cerebroside Sulfate

Kenji WAGO, M. D.

Cerebroside preparations were administered orally or intravenously to cholesterol-fed rabbits. The oral administration of bovine brain extracts, namely, crude and purified sphingolipids, proved to alleviate the diet-induced hypercholesteremia. Intravenous administration of cerebroside and its sulfuric ester also inhibited the elevation of serum total lipid, total cholesterol, C/P ratio, and β/α lipoprotein index, and mitigated the atheromatous involvements in the aorta. Cerebroside sulfate, further, showed anticoagulant activity, and seemed to increase the excretion of digitonin-precipitable, Liebermann-Burchard-positive fecal sterols. Possible mechanisms for these effects are discussed.

IN 1908, Dorée and Gardner[1] found that a dog on raw brain diet excreted large amounts of coprosterol in the feces, while the fecal sterols exclusively consisted of cholesterol when the dog was fed various mixed diets of meat and vegetables. About 30 years later, Rosenheim and Webster[2] investigated this observation and studied a relationship between diet and coprosterol formation in an experiment with rats. On the sterol-free standard diet the rats excreted only traces of coprosterol. When cholesterol was added to the diet, it was excreted unchanged. On replacing a part of the cholesterol by cooked brain, however, large amounts of coprosterol appeared in the feces. Hence, they presumed that brain might contain a substance or factor which is essential for the intestinal conversion of cholesterol into coprosterol, and confirmed that Thudichum's white matter was rich in the factor, attributing the specific action to a cerebroside, phrenosine. In fact, rats on a high cholesterol diet, with the addition of phrenosine or cholesterol-free brain extract, excreted coprosterol to the extent of 70 to 90% of the fecal sterols.

Furthermore, Prange and his associates[3] recently observed that the conversion of cholesterol into coprosterol was carried out in vitro by bacteria, cultivated from human feces, to the extent of 90% in a medium

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containing as little cerebroside as 0.174 micrograms per test tube (9 ml.).

From these experiments it was suggested that, if cerebroside could convert intestinal cholesterol, both of dietary and biliary origin, into unabsorbable coprosterol in the alimentary canal, body cholesterol could be removed via the gastrointestinal tract. Jones and co-workers[4)-8)] in their serial studies demonstrated that the oral administration of cholesterol-free mammalian brain extract, rich in cerebroside, actually reduced the serum cholesterol level in chicks and men, and that the effectiveness closely resembled that of β-sitosterol. In 1957, Rolfo et al.[9)] reported the effect of a mixture of cerebroside and sphingomyelin against diet-induced hypercholesteremia in dogs.

Another explanation on the mechanism of this substance for hypocholesteremic effect is made by Carroll[10),11)] who found that the feeding of cerebroside or fatty acids from the glycolipid increased the excretion of fecal sterols in rats, and identified the greater part of the sterols with cholesterol on chromatographic assay, regarding the cholesterol as 'endogenous,' namely as synthesized in the liver.

In this respect, however, Jones[12)] himself later corrected his original presumption. In 1957, he and his associates sustained that the turnover rate of the serum cholesterol in dogs and chicks, as measured by biological decay of endogenously labelled C^{14} cholesterol, was substantially increased by the feeding of a cerebroside-rich extract of mammalian brain. And in hypercholesteremic patients, where the rate of synthesis of cholesterol (estimated from incorporation of intravenously administered C^{14} acetate) was not greatly changed, the turnover rate of the serum cholesterol was elevated and the total steroid excretion in the feces was increased by this agent. These recently undertaken studies suggest that the elevated turnover rate of serum cholesterol may be produced by an increased fecal excretion of endogenous cholesterol.

As to anticoagulant activity, Chargaff[13)-15)] reported in 1937 that cerebroside fractions from mammalian brains acted as an inhibitor of the blood clotting, succeeded in extracting the inhibitor from sheep brain, and finally confirmed that synthetic cerebroside sulfuric acids had a marked inhibitory effect upon the clotting of blood and plasma while cerebrosides themselves were inactive in coagulation mechanism.

Recently sphingosine, base from cerebroside, has been regarded as antithromboplastic factor by some investigators. Hecht[16)-18)] discovered in 1951, in the course of a study of his non-protein activator, 2 new inhibitors of the first stage of the clotting process, namely, glutamic acid and sphingosine. Both compounds were completely ineffective in the second stage of coagulation.

The experiments to be reported here mainly concerned the inhibi-

tory effect on atherosclerosis and coagulation of a cerebroside sulfate preparation.

Materials and Methods

Oral Administration of Sphingolipid Preparations

The author preliminary studied serum cholesterol reduction by the oral administration of 3 different sphingolipid preparations viz., crude cerebroside, purified cerebroside and sphingomyelin.

Six-month-old male rabbits of the average weight of 2.2 Kg. obtained from a commercial breeder were caged individually, divided into 4 groups, and fed a cholesterol-poor commercial standard diet. The control group (I) was given the standard diet mixed with 100 mg. of cholesterol per Kg. of body weight per day. Group II and III received supplements of crude cerebroside 250 mg., purified cerebroside 100 mg., and sphingomyelin 100 mg., respectively, per Kg. of body weight per day. They were kept on the diets for 12 weeks and then sacrificed for pathological investigation. Blood samples were drawn from the ear vein before initiating the feeding and every 2 weeks during the period of the administration, and serum cholesterol was determined by the method of Sperry-Webb.[19] Each rabbit was weighed bi-weekly and blood clotting time was measured by Sahli-Fonio method.

These three sphingolipid fractions were prepared by the extraction of Ceremon, commercial beef brain hydrolysate obtained by enzymatic procedure with ground pig pancreas.

Five kilograms of Ceremon, which contains about 10% cerebroside, was mixed with 11 L. of acetone and kept at boiling temperature for 5 hours, and the extract was removed by filtration. The residue was extracted with hot ethanol (at 78°C) for 4 hours and filtered rapidly. The filtrate was allowed to settle at room temperature until precipitation was almost completed. The precipitate was recrystallized from alcohol and dried. The fraction thus obtained weighing 180 Gm. consisted of 80% cerebroside, as calculated from galactose content (determined as hexose by the method of Hagedorn-Jensen), and presumably sphingomyelin, as the greater part of the residue. Analysis found N 3.0%, P 1.3% and S 0.4% (determined by the method of Tanaka[20]). This is the crude cerebroside preparation in the present paper from which both purified cerebroside and sphingomyelin were obtained.

The crude cerebroside powder was dissolved with glacial acetic acid at boiling temperature. The solution deposited on cooling a large amount of cerebroside precipitates, which were separated by filtration and desiccated. Purified cerebroside fraction, thus obtained, contained over 95% cerebroside. Analysis found galactose 22.6%, N 2.1% and P 0.5%. Sphingomyelin was precipitated by concentrating the filtrate of the above-mentioned acetic acid solution and recrystallized from petroleum-ether-alcohol (9 : 1). This fraction contained, on analysis, 3.9% N and 2.6% P.

Intravenous Administration of Cerebroside and its Sulfuric Ester

Two different cerebroside preparations were administered intravenously in

the main experiment, namely, 1% cerebroside suspended in distilled water and 2% cerebroside sulfate in 0.85% saline. In this experiment, rabbits were divided into 3 groups. The control group (A) was kept on the same diet as in the preliminary experiment. Group B and C, in addition, received an intravenous injection of cerebroside and its sulfuric ester, respectiviely, in dose of 10 mg. each per rabbit every second day. Blood samples were obtained biweekly for the determination of serum total lipid, total cholesterol, phospholipid and β/α lipoprotein ratio. Total lipid was determined by a turbidity method,[21] total cholesterol by a modified method of Zak,[22] lipid phosphorus by the method of Allen,[23] and lipoprotein ratio by the method of Swahn[24] after electrophoretic treatment of the samples.

The rabbits were sacrificed 12 weeks after initiating the administration of the substances, the aortae were opened, and the aspects of internal surfaces were described. The degree of atheromatous involvement was graded on an arbitrary scale of 0 to 4+ : no involvement 0, fatty streak 1+, 2+, fibrous plague or atheroma 3+, 4+. Then, sections from the aortae were microscopically investigated. As survey stain for fatty infiltration, Sudan III stain was used, and for mucopolysaccharide research, PAS and toluidine blue stains were applied. Cerebroside in the tissue was examined by the technique of Okamoto et al.[25]

As to the preparation of cerebroside and cerebroside sulfate suspension, the former was simply made by suspending purified cerebroside in distilled water, while the latter was obtained from beef brain by the method of Folch et al.[26] Analysis for terminal product, found galactose 19.5%, (by a carbazol method[27]), P 0.0013%, S[20] 3.4%, hexosamine 1.1% (by the method of Elson-Morgan[28],[29]), and sialic acid 4.0% (by a Bial method[30]). The sulfur content suggests that the greater part of this fraction might be monosulfuric ester of cerebroside. (The position of SO_4 group was shown to be the primary hydroxyl of carbon atom 6 of the galactose moiety of the molecule by Thannhauser[31]). The small amount of hoxosamine and sialic acid indicate the possible contamination of ganglioside. The term 'cerebroside sulfate' denotes this fraction throughout this paper.

Effect of Cerebroside Preparation on Fecal Sterol Excretion

An attempt was made in an additional experiment, to study the relationship between serum cholesterol level and fecal sterol excretion during the period of cerebroside administration. Special rabbit cages were provided for the purpose of separating feces from urine. Rabbits were divided into 8 groups and kept in the conditions listed in Table II for 7 days. Serum total cholesterol was determined before and immediately after the treatment. Feces excreted during the period were collected and weighed. And the fecal sterols were measured by the method outlined by Carroll.[11]

Effect of Cerebroside Sulfate on the Blood Clotting

Since it was observed that the blood clotting time was prolonged to some extent after the intravenous administration of cerebroside sulfate in the above-mentioned main experiment, its effect on blood coagulation was studied.

The experiment consisted of 7 tests; (1) recalcification time, (2) throm-

boplastin generation test, (3) prothrombin time, (4) thrombin-fibrinogen reaction (5, 6) influence of DFP (defibrinated plasma) and Polybrene on the activity of the sample, and (7) influence of the sample on fibrinolysis. Precise methods for each test are described on the footnotes of Tables and Figures.

Clinical Observation

Subjects were selected from in- and out-patients of the Tokyo University Hospital. The diseases of the patients, sex, age, and the dosage of the drug are listed in Table VII. Cerebroside was administered in the form of tablet. Each tablet contained 300 mg. of the substance. Serum total cholesterol of the patients was determined by a Kiliani method[22] at the Central Laboratory of the Hospital.

Results

Oral Administrations of Sphingolipid Preparations

Fig. 1 shows an inhibitory effect of sphingolipids on dietary induced hypercholesteremia in the rabbit. Mean serum total cholesterol values were significantly reduced in Group II fed crude cerebroside, presumably consisting of a large amount of cerebroside and small amounts of sphingomyelin, in comparison to the control levels. Both Group III and IV showed similar but less reduction of total cholesterol. There was no

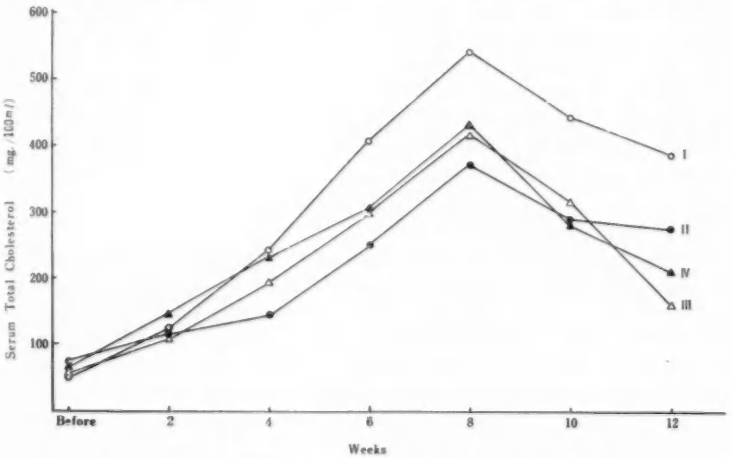

Fig. 1. The average values of serum total cholesterol in different dietary groups during the period of observation.

Group	Diet	No. of Rabbits
I	Standard Diet (SD)+Cholesterol (Ch)	17
II	SD+Ch+Crude Cerebroside	6
III	SD+Ch+Purified Cerebroside	12
IV	SD+Ch+Sphingomyelin	4

Table I. The Chemical Findings of Serum Lipids Before and During the Intravenous Administration of Cerebroside Preparations and the Degrees of Atheromatous Involvements Found at Autopsy*

Group	Diet and injection Treatment	Number of rabbits	Sort of serum lipid research	Before	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks	Degree++++ of atheromatous involvement
A	Standard diet (SD)+ Cholesterol+ (Ch) No injection	13	Total lipid (mg./100 ml.)	366±57						959±164	2.8
			Total cholesterol (TC) (mg./100ml.)	74±17	186±55	351±99	490±126	533±125	460±129	411±115	
			Cholesterol-phospholipid+++ ratio (C/P)	0.46±0.11	0.88±0.22	1.41±0.33	1.70±0.45	178±0.45	1.93±0.44	1.64±0.30	
			β/α lipoprotein index	1.6±0.2	2.2±0.4	3.0±0.6	3.9±0.2	4.5±1.0	4.1±1.2	4.1±0.9	
B	SD+Ch Cerebroside,++ intravenous	6	TL (mg./100 ml.)	321±25						792±318	2.4
			TC (mg./100 ml.)	71±18	164±52	292±159	372±128	351±150	260±137	233±117	
			C/P	0.43±0.09	0.75±0.26	1.23±0.38	1.27±0.43	1.11±0.40	1.13±0.30	0.89±0.34	
			β/α	1.5±0.2	1.9±0.5	2.4±0.5	2.9±0.9	3.6±1.7	2.4±1.0	2.5±0.8	
C	SD+Ch Cerebroside sulfate,++ intravenous	8	TL (mg./100 ml.)	340±52						733±140	1.7
			TC (mg./100 ml.)	76±17	156±25	283±74	334±78	421±95	376±84	218±88	
			C/P	0.46±0.06	0.75±0.11	0.94±0.15	1.09±0.10	1.10±0.25	1.24±0.31	1.20±0.44	
			β/α	1.8±0.2	2.2±0.2	2.8±0.8	3.3±0.8	3.8±0.8	3.0±0.9	2.4±0.6	

* Values denote Average±Standard Deviation.

\+ Cholesterol was administered at the rate of 100 mg. per Kg. of body weight per day.

++ Each rabbit of Group B and C received 10 mg. of cerebroside and cerebroside sulfate respectively every second day.

+++ Phospholipid was calculated as lecithin from lipid phosphorus determined by the method of Allen.

++++ Degree of atheromatous involvement was graded on an arbitrary scale of 0 to 4+.

remarkable difference in serum cholesterol ester ratio, weight gain or blood clotting time between the control and the experimental groups. Atheromatous lesions in the aorta were more marked in the control group than in the sphingolipid-fed groups.

Intravenous Administration of Cerebroside and its Sulfuric Ester

Table I. presents the chemical findings before and during the intravenous administration of cerebroside preparations and the degrees of atheromatous involvements found at autopsy for each group. The mean values of serum total lipid, total cholesterol, C/P ratio and β/α lipoprotein index for the treated groups B and C were significantly lower than those of the control group A. Gross pathological findings showed that atheromatous lesions were obviously inhibited in the rabbits given cerebroside sulfuric ester, as compared with the involvements in the control rabbits. Group B presented less severe lesions in the aorta than Group A, though the lesions were more marked than Group C. Histochemically, there were no qualitative difference in the staining properties of lipids and mucopolysaccharides deposited in the atheromatous area of the aortic tissues among these 3 groups. Cerebroside infiltration could not be detected in the involved aorta of each group.

Effect of Cerebroside Preparations on Fecal Sterol Excretion

The amounts of digitonin-precipitable, Liebermann-Burchard-positive fecal sterols excreted by rabbits of 8 groups are listed in Table II,

Table II. Effect of Cerebroside Preparation on Fecal Sterol Excretion in the Rabbit*

Group	Diet and treatment+	Number of rabbits	Fecal sterol++ (mg./rabbit/day)			Serum total cholesterol (mg./100ml.)					
						Before			After		
I	Standard diet (SD)	3	41	26	54	94	58	82	103	52	81
II	SD+Cerebroside, fed	3	94	66	21	76	88	63	78	91	54
III	SD+Cerebroside, inj.	3	18	73	38	80	74	73	79	69	73
IV	SD+Cerebr. Sulf., inj.	3	49	103	64	76	98	49	72	90	63
V	SD+Cholesterol (Ch)	3	176	211	223	68	71	76	138	134	105
VI	SD+Ch+Cerebr., fed	3	246	103	152	83	79	70	120	99	123
VII	SD+Ch+Cerebr., inj.	3	189	97	257	77	83	90	86	151	122
VIII	SD+Ch+Cerebr. sulf.	3	241	264	276	65	70	79	98	104	95

* Each treatment was carried out for 7 days.

+ Standard diet was cholesterol-poor commercial one
Dose of supplements was as follows (each per rabbit, per day);

Cholesterol, fed	300 mg.,	Cerebroside, fed	1.0 Gm.
Cerebroside, inj.	20 mg.,	Cerebr. sulfate, inj.	20 mg.

++ Values for fecal sterol denote the amounts of digitonin-precipitable, Liebermann-Burchard-positive sterols excreted.

together with the diets and treatments given to these groups and serum total cholestrol values just before and after the treatments. It is likely that intravenous injection of cerebroside sulfate might have increased the fecal sterol excretion. However, it can not be definitely proved because of the limited number of cases.

Anticoagulant Activity of Cerebroside Sulfate

Recalcification time was markedly prolonged by adding cerebroside sulfate to reaction mixture (Table III). Thromboplastin generation was

Table III. Effect of Cerebroside Sulfate on Recalcification Time*

Cerebroside sulfate (%)+	0	0.02	0.08	0.1
Recalcification time	2'45"	5'20"	5'00"	6'10"

* Reaction mixture for measuring recalcification time.

Oxalated plasma	0.1 ml.
Cerebroside sulfate suspended in 0.85% saline	0.1 ml.
M/40 $CaCl_2$	0.1 ml.

+ Each percentage in the table denotes the concentration of cerebroside sulfate in the saline suspension.

significantly inhibited by the substance even at a concentration of 0.004% in incubation mixture (Fig. 2). Prothrombin time was not prolonged by the sphingolipid at the concentration of approximately 0.003% in reaction mixture, while higher percentage of this drug caused a marked prolongation (Table IV). Thrombin-fibrinogen reaction was inhibited by the

Table IV. Effect of Cerebroside Sulfate on Prothrombin Time*

Cerebroside sulfate (%)+	0	0.02	0.06	0.1	0.2	0.3	0.5	0.1
Prothrombin time (sec.)	18	18	18½	20	20½	25½	34	52

* Reaction mixture for measuring prothrombin time.

Oxalated plasma	0.1 ml.
Rabbit-brain-thromboplastin	0.1 ml.
M/40 $CaCl_2$	0.1 ml.
Cerebroside sulfate suspended in 0.85% saline	0.05 ml.

+ Each percentage in the table denotes the concentration of cerebroside sulfate in the saline suspension.

glycolipid as shown in Table V, and the antithrombin activity was completely extinguished by Polybrene (hexadimethrin bromide), exclusively

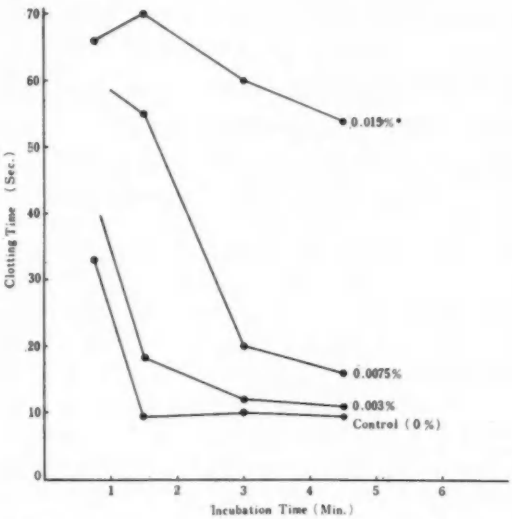

Fig. 2. Inhibiting effect of cerebroside sulfate on the first stage of the blood clotting.

Tricalcium-phosphate-gel-absorbed plasma (5× diluted with saline) 0.2 ml.,
Platelet suspension 0.2 ml.,
Serum (0.1 ml.)+Cerebroside sulfate+0.85% NaCl 0.2 ml.,
and M/40 $CaCl_2$ 0.2 ml.
were mixed and incubated for 1 to 6 minutes.

One-tenth ml. from each incubation mixture, oxalated plasma 0.1 ml. and M/40 CaCl were mixed and the clotting time was measured.

* Values for the concentrations of cerebroside sulfate in the incubation mixtures are noted at the right of the figure.

antiheparinic substance. Moreover, this substance presented toluidine blue metachromasia at pH 2.5 in the same color and in the same degree as heparin. These observations were associated with the report by Green and Robinson[32] that a mast cell tumor of the mouse contained cerebroside sulfate and that the compound turned over very rapidly in the mast cells. But addition of defibrinated plasma (DFP) to cerebroside sulfate, contrary to the addition of DFP to heparin, incited diminution of anticoagulant activity (Table VI). As to the influence of the sulfolipid on fibrinolysis, any definite accelerating or inhibiting activity was not proved so far.

Table V. Effect of Cerebroside Sulfate on Thrombin-Fibrinogen Reaction*

Cerebroside sulfate (%)+	0	0.075	0.01	0.015	0.075	0.15
Clotting time (sec.)	14	14	14½	15	50	100

* Mixture for measuring clotting time in thrombin-fibrinogen reaction.

Fibrinogen (fractionated by ammonium sulfate)	0.1 ml.
Cerebroside sulfate suspended in veronal-buffered isotonic saline (pH 7.3 μ 0.15)	0.1 ml.
Thrombin (7.5 μ/ml.)	0.1 ml.

+ Each percentage in the table denotes the concentration of cerebroside sulfate in the VBIS-suspension.

Table VI. Influence of Defibrinated Plasma (DFP) upon the Antithrombic Activity of Cerebroside Sulfate*

Cerebroside sulfate	(−)	(+)					
DFP (ml. in VBIS susp. 0.1 ml.)	(−)	0	0.02	0.04	0.06	0.08	0.1
Clotting time (sec.)	9	16½	13	11	10½	10½	9

* Mixture for measuring clotting time in thrombin-fibrinogen reaction with and without DFP.

Fibrinogen	0.1 ml.
Thrombin (7.5 μ/ml.)	0.1 ml.
Cerebroside Sulfate (0.03%)	0.1 ml.
DFP diluted with VBIS (pH 0.3)	0.1 ml.

Clinical Studies

One half of the 6 patients were in-patients with disease of central nervous system and the other half were out-patients with hypertension. They were treated with the previously mentioned cerebroside tablets at least for one week. Every subject showed reduction in serum total cholesterol by the administration of the drug as shown in Table VII, and, in a case of hereditary cerebellar ataxia, disturbance of the gait was remarkably alleviated.

Table III. Effect of a Cerebroside Preparation on the Serum Cholesterol of Six Patients*

Patient	Sex	Age	Disease	Daily dose	Duration of administration	Serum cholesterol (mg./100ml.)+	
						Before	After
Y. K.	M	56	Oligo-ponto-cerebellar atrophy, suspected	2.7Gm.	2 Months	265	230
M. O.	M	44	Multiple sclerosis, suspected	1.8	2 Weeks	308	225
S. N.	M	43	Hereditary cerebellar ataxia	2.7	1 Week	260	225
R. S.	M	51	Cerebral arterio-sclerosis	2.7	1 Month	241	190
K. K.	F	58	Hypertension	1.8	3 Weeks	237	212
K. K.	F	57	Hypertensive ence-phalopathy	2.8	3 Weeks	267	235

* The preparation was supplied in the form of tablet by Teikoku-Zoki Co., Ltd. Each tablet contains 300 mg. of cerebroside.

\+ Serum total cholesterol was determined by a modified method of Zak at the Central Laboratory, Tokyo University Hospital.

Discussion

As previously mentioned, the hypocholesteremic effect of cerebroside preparations has been studied by some investigators in the past decade and applied to the treatment for atherosclerosis. However, it still remains unsolved (a) whether cerebroside alone in the brain extracts is essential for the anti-atherosclerotic activity or not (in other words, whether the presence of sphingomyelin or other factors is necessary or not), (b) how anti-atherosclerotic effect comes when cerebroside is administered intravenously, and (c) what is the satisfactory explanation for the mechanism of the hypocholesteremic effect of this drug.

(a) Jones' mammalian brain extract consisted of 57% cerebroside, 41% sphingomyelin and other substances.[7] In the sphingolipid mixture administered by Rolfo to dogs, the proportion of cerebroside to sphingomyelin was about 4 to 6.[9] The sugar content of Carroll's cerebroside fractions ranged from 19 to 7% and their phosphorus content varied from 0.7 to 2.6%,[11] while purified cerebroside fraction used in the present experiment contained 22.6% galactose and 0.5% P (Theoretical value for galactose content of cerebroside is approximately 22%, and phosphorus 0). In the preliminary experiment, the result gave no evidence that sphingomyelin should be indispensable for the hypocholesteremic effect. But it is probable that the addition of sphingomyelin to cerebroside

might promote the inhibitory effect on atherosclerosis.

(b) Results in the main experiment established the effect of cerebroside against diet-induced hypercholesteremia in the rabbit by intravenous administration.

(c) And further, these results seem to corroborate a recent hypothesis on the antilipemic mechanism of cerebroside. Originally, the hypocholesteremic activity appeared to depend on the ability of this drug to convert intestinal cholesterol into unabsorbable coprosterol. Recently, however, a presumption has been becoming predominant that the elevation of turnover rate of cholesterol through excretion of 'endogenous' cholesterol may account for the effect.[11),12)] Results in the additional experiment, comparable to values obtained by Carroll,[11)] seemed to substantiate his opinion partly that the increased fecal sterol excretion during the period of cerebroside administration may originate from endogenous cholesterol. But further studies of a larger scale, including more precise chemical assay of steroids, are desirable for giving a definite answer to the complicated problem of 'absorption and excretion of cholesterol'.

Aside from the above-discussed influence of cerebroside upon cholesterol metabolism, pathological and biochemical role of sphingolipids in atheroma formation, especially its direct participation in the arterial wall lesion, has not been well deliniated. Murakami[33)] reported that sphingomyelin content increased in the atheromatous aorta, and Schönheimer[34)] detected, protagon or sphingolipid in the aortic wall with atheromatous involvements. Cerebroside deposition, however, could not be found in the involved aorta from rabbits used in the present study by the histochemical technique of Okamoto.[24)] Studies on anticoagulant activity of cerebroside sulfate indicated that there might be 2 processes in the inhibitory effect of the substance on the blood clotting, namely, activity inhibiting thromboplastin generation and activity inhibiting thrombin-fibrinogen reaction. The former seems to be related to the fact confirmed by Hecht that sphingosine, a component of both cerebroside and sphingomyelin, acts as inhibitor of the first stage of coagulation, not as inhibitor of the second stage.[17)] The latter appears to be connected with antithrombic activity of 'sulfated' mucopolysaccharides such as heparin and chondroitin polysulfate. And it appears very interesting that both cerebroside and sphingomyelin, containing sphingosine and no sulfuric radical have never showed anticoagulant activity.[15)-17)] In the clinical studies, it seems to be worthwhile to note that relatively small amounts of cerebroside (1.8 to 2.7 Gm. per day) was effective, as compared to large amounts of brain extract (30 Gm. per day, cerebroside 57%) administered by Jones.[6),7)]

The improvement of nervous function in a patient with cerebellar ataxia calls attention to the observation reported by Noble et al.[35] that, during the feeding of fatty acids from cerebrosides, 6 patients with disseminated sclerosis showed no exacerbation. He and his associates presumed that the maintenance and function of myelin sheath of nerves might be dependent on the specific nature of the fatty acid components of cerebrosides. In this field of cerebroside research, also, further investigations are highly desirable.

Experimental and clinical studies on cerebroside and its closely connected substances are still in progress.

Summary

(1) Oral administration of crude cerebroside extracted from bovine brain inhibited the diet-induced elevation of serum cholesterol in the rabbit. Both purified cerebroside and sphingomyelin showed similar and less effect.

(2) Intravenously administered cerebroside and cerebroside sulfate proved to have hypocholesteremic effect. The latter significantly inhibited atheromatous involvements in the aorta.

(3) Cerebroside sulfate increased fecal excretion of digitonin-precipitable, Liebermann-Burchard-positive sterols by intravenous administration.

(4) Cerebroside sulfate inhibited both thromboplastin generation and thrombin-fibrinogen reaction.

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Experimental Studies on the Nervous Control of the Renal Circulation—Effect of the Electrical Stimulation of Splanchnic, Vagal and Other Somatic Nerves and of the Carotid Sinus Reflex on the Renal Circulation (II)

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The renal blood flow was measured by an electromagnetic flow meter in dogs with the innervated kidney in situ perfused by the donor's blood. This perfusion was designed in order to avoid the possible influence of humoral factors and of prerenal circulatory changes on the nervous control of renal hemodynamics in studied dogs. It was confirmed that stimulation of the distal cut end of the splanchnic nerve resulted in marked renal vasoconstriction. And slight renal vasoconstriction was also observed in the stimulation of the distal end of the divided vagal nerve. By occlusion of carotid arteries, slight renal vasoconstriction was observed. It was in contrast to the independence of renal vascularity when a pressure was given on the carotid sinus. Responses of renal blood vessels were variable and not consistent in the stimulation of the proximal end of the divided femoral and sciatic nerve, as well as in the stimulation of the proximal end of the divided vagal nerve.

IN a previous paper,[1] we reported some results of the experimental studies on the nervous control of the renal circulation, especially on effects of the electrical stimulation of splanchnic, vagal and other somatic nerves and of the occlusion of carotid arteries in dogs. But with the techniques described previously, the influence of humoral factors and of prerenal circulatory changes, such as in cardiac output, might have been partly responsible for the alteration of renal hemodynamics. Therefore, an improvement for the experimental technique and devices were necessitated in order to measure the neural control on the renal vessels more exactly.

In the present study, the innervated kidney was perfused in situ by the donor's blood which was kept independent of stimulating procedure;

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thus effects of such factors as prerenal circulatory changes and of humoral agents could be avoided throughout the experiment.

Methods

Experiments were performed on 21 dogs each weighing 9.6 to 15.0 Kg. Anesthesia was made with thiopental sodium (20 to 30 mg./Kg.) in 13 dogs, with pentobarbital sodium (5 to 10 mg./Kg.) in 5, and with chloralose (50 to 80 mg./Kg.) plus morphine chloride (6 to 4 mg./Kg.) in 3. In each experiment, another dog as large as the experimental animal (recipient) was used as a donor to perfuse the recipient's kidney. After a donor was heparinized, its femoral artery was connected with recipient's renal artery and the renal vein of the recipient was connected with donor's femoral vein through an electromagnetic flow meter. The connections were made by polyethylene tubes as closely as possible. Thus in this procedure, which we described in the preliminary reports,[2), 3)] the kidney was perfused completely by donor's blood during the experiment with a stable pressure which was recorded by an electromanometer connected to a side-branch in the perfusing circuit.

The nerve stimulation and the carotid occlusion were carried out in similar fashions as described previously.[1)] In addition, a blind sac was made on the unilateral carotid artery and a high pressure was given thereupon according to the method described by Moissejeff,[4)] and effect of carotid sinus reflex on renal circulation was investigated.

Results

Splanchnic Nerve Stimulation—

Tirty-five electrical stimulations of the distal end of the divided greater splanchnic nerve were made on 4 dogs. A rise in systemic arterial pressure and a simultaneous decrease in renal blood flow occurred in all 29 experiments except 6 instance with inadequate stimulating conditions (Fig. 1). The elevation of mean arterial pressure ranged from 7 to 53 mm. Hg with an average of 21 mm. Hg (4 to 41.4%; mean 15%). The decrease in reanl blood flow was 11 to 62 ml./min. with a mean of 37 ml./min. (17 to 100%; mean 53%).

Hydergine® was given intravenously prior to nervous stimulations in 2 dogs. The vosoconstrictive responses were inhibited or supressed in all 6 experiments on these dogs. No evidence for the vasodilatatory effect of nervous stimulation was observed (Fig. 2).

Efferent Vagal Nerve Stimulation—

Stimulation of the distal end of the cut cervical vagal nerve produced a prompt fall in systemic arterial pressure with cardiac arrest or brady-

cardia and a slight decrease in renal blood flow in all 5 experiments on 4 dogs (Fig. 3). The decrease in renal blood flow ranged from 3 to

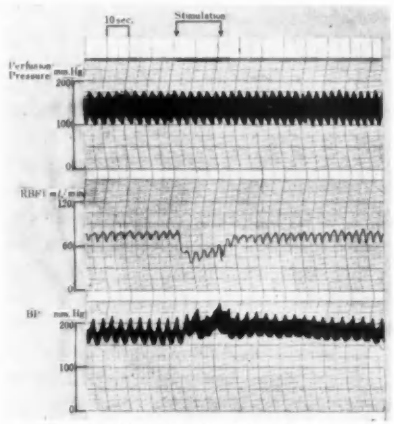

Fig. 1. Dog No. 95, Exp. 9: Stimulation of distal end of divided greater splanchnic nerve (100 c/s, 3 v).

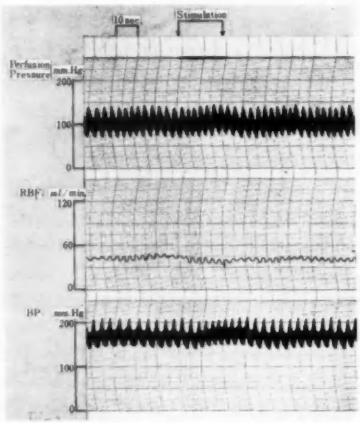

Fig. 2. Dog No. 95, Exp. 17: Stimulation of distal end of divided greater splanchnic nerve (30 c/s, 5 v) after intravenous injection of 0.9 mg. of hydergine.

23 ml./min. with an average of 13 ml./min. (21 to 38%; mean 22%). In all experiments except one, decrease in renal blood flow was over 11 ml./min., which could be regarded as significant.

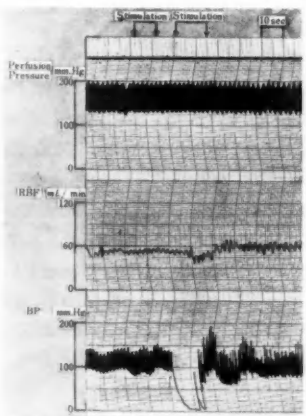

Fig. 3. Dog No. 93, Exp. 3 and Exp. 4: Stimulations of distal end of divided cervical nerve (30 c/s, 3 v and 50 c/s, 5 v).

Occlusion and Giving Pressure on Carotid Artery—

The occlusion of bilateral or unilateral common carotid arteries was followed by an elevation of systemic arterial pressure, ranging from 11 to 35 mm. Hg, with an average of 22 mm. Hg (9 to 26%; mean 17%). In 9 of 12 experiments on 5 dogs, pressor responses accompanied by more or less decrease in renal blood flow, ranging from 3 to 9 ml./min. with an average of 5 ml./min. (3 to 23%; mean 12%). But significant decrease was found in only 4 experiments among them (Fig. 4).

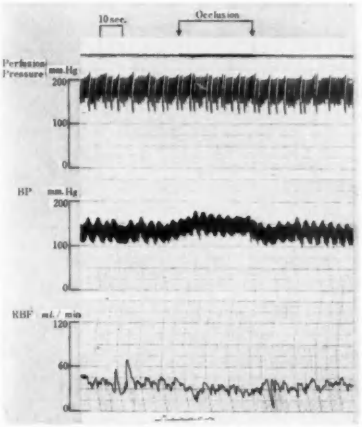

Fig. 4. Dog No. 133, Exp. 14: Occlusion of unilateral common carotid artery.

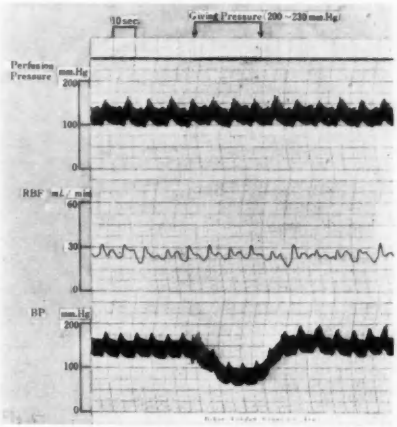

Fig. 5. Dog No. 132, Exp. 3: Giving pressure unilaterally on blind sac prepared from carotid artery including carotid sinus.

When a high pressure (160 to 220 mm. Hg) was given into a blind sac, prepared on a common carotid artery, a fall of systemic arterial pressure was observed in association with bradycardia but the renal blood flow remained unchanged. Decrements of systemic arterial pressure in 11 experiments on 3 dogs were 9 to 62 mm. Hg with a mean of 36 mm. Hg (Fig. 5).

Afferent Stimulation of Sciatic or Femoral Nerve—

Stimulation of the proximal end of the divided sciatic or femoral nerve in 12 dogs caused both hypertensive and hypotensive responses. Significant decrease in renal blood flow was observed in 13 experiments on 6 dogs. Blood pressure responses in these 13 experiments were hypertensive in 5 experiments, hypotensive in 5, and no significant change in blood pressure was observed in the remaining 3. Fig. 6 and Fig. 7 show a decrease in renal blood flow with hypotensive response in sciatic nerve

stimulation and with hypertensive response in femoral nerve stimulation respectively. Among other 25 experiments with significant alterations in systemic blood pressure, renal blood flow decreased merely slightly (3 to

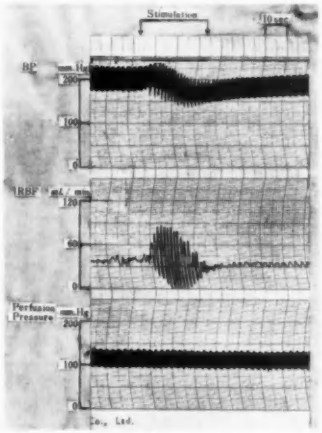

Fig. 6. Dog No. 104, Exp. 5: Stimulation of central end of divided sciatic nerve (100 c/s, 5 v).

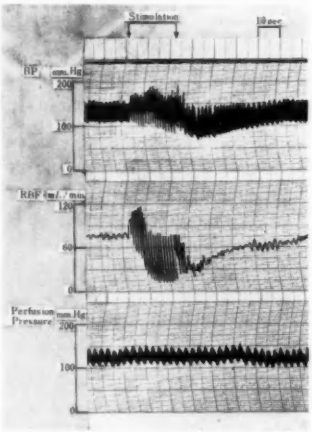

Fig. 7. Dog No. 99, Exp. 1: Stimulation of central end of divided femoral nerve (50 c/s, 5 v).

7 ml./min., not significant) in 18 and did not change in 7. Increase in renal blood flow (8, 9, 12 and 21 ml./min., respectively) occurred in 4 experiments, 2 of which were repeated in the same dog and resulted in slight hypertensive responses (Fig. 8); 2 experiments in another dog produced hypotensive responses.

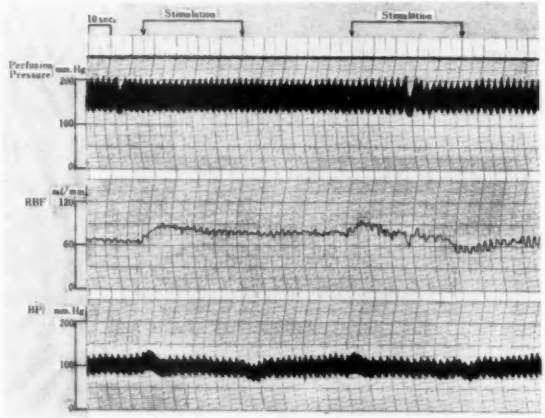

Fig. 8. Dog No. 93, Exp. 1 and 2: Stimulations of central end of divided femoral nerve (30 c/s, 5 v).

Afferent Cervical Vagal Nerve Stimulation—

A total of 86 experiments were performed on 14 dogs. Systemic arterial pressure responses produced by stimulation of the central end of the divided cervical vagal nerve were not consistent, that is, hypertensive in 46, hypotensive in 8 and biphasic in 2 experiments. The alteration in renal blood flow was also variable. In 46 hypertensive responses, the renal blood flow significantly increased in 7 (8 to 14 ml./min.), decreased in 11 (8 to 51 ml./min.) and did not change in 28 experiments. In 8 hypotensive responses, the renal blood flow significantly increased in 4 (8 to 18 ml./min.), decreased in one (8 ml./min.) and did not alter in 3 experiments. Fig. 9 is an example which shows a decrease in renal blood flow with a hypertensive response and Fig. 10 shows a slight

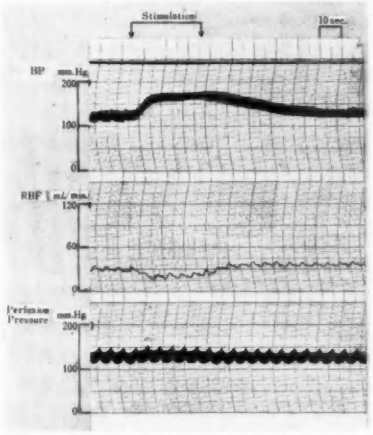

Fig. 9. Dog No. 109, Exp. 9: Stimulation of central end of divided cervical vagal nerve (100 c/s, 20 v).

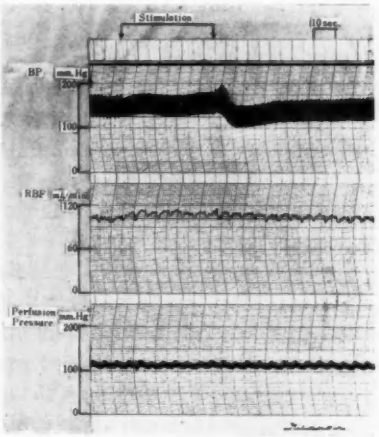

Fig. 10. Dog No. 108, Exp. 9: Stimulation of central end of divided cervical vagal nerve (100 c/s, 20 v).

increase in renal blood flow accompanied by a hypertensive response. No significant change in renal blood flow occurred in experiments with biphasic blood pressure responses. One significant decrease and one significant increase in renal blood flow were observed in 30 experiments without significant alteration in blood pressure.

In 3 of the 14 dogs, stimulation in low voltage (2 to 5 volts) appeared to produce an increase in renal blood flow with a hypotensive response, whereas that in high voltage (5 to 20 volts) resulted in a decrease in renal blood flow with a hypertensive response.

Discussion

We have been interested in the experimental studies on the regulatory mechanisms of renal circulation under the nervous control. In a previous paper,[1] we reported some results of our experimental studies in which effects of the electrical stimulation of splanchnic, vagal and other somatic nerves and of the carotid occlusion on renal circulation were observed in dogs. Changes in renal blood flow were measured by an electromagnetic flow meter which was set between renal and femoral veins of the studied dog. But with the techniques described previously, possibility remained that renal hemodynamics might have been influenced by humoral factors such as epinephrine[5),6)] released from any parts of the body and by prerenal circulatory changes[7),8)] such as in cardiac output.

In the present study, the renal blood flow was measured on the innervated kidney in situ perfused by donor's blood. Since a donor dog was kept independent of any stimulating procedures, the perfusing pressure was maintained constant throughout the experiment. Thus, effects of such factors as prerenal circulatory changes and of humoral agents could be avoided. Then, alterations in the recorded renal blood flow could be considered as indicating the attitude of renal vessels more directly and exactly.

On splanchnic nerve stimulation, the results reported previously were confirmed in this perfusing method; the elevation in systemic arterial pressure and the marked reduction in renal blood flow indicated vasoconstriction of the renal blood vessels. The result supports the previously presumed conclusion that the decrease in renal blood flow might be attributed to a direct neural effect rather than to a humoral effect, since the perfused kidney was isolated from the recipient's blood.

In the previous study, the renal blood flow decreased by stimulating the distal end of the cut subdiaphragmatic vagal nerve. But changes in the calculated renal vascular resistance were not significant. In the present study, the renal blood flow decreased in all experiments. This fact indicates that the renal vasoconstriction due to neural impulses occurs by stimulation of the distal end of the cut cervical vagal nerve, because the perfusing arterial pressure was constant during experiment. It is not clear, however, whether this renal vasoconstriction was due to direct stimulation of cervical vagosympathetic trunks or due to a reflex secondary to a cardiac effect.

On carotid occlusion, the slight decrease in renal blood flow which is considered to be due to renal vasoconstriction was observed, although significant decrease in renal blood flow was recorded in 4 among 12

significant hypertensive responses. This appears to support our previous findings that the calculated renal vascular resistance increased slightly in all cases.

Sollmann and Brown[10] observed a diminution in volume of the perfused innervated kidney by means of oncometric tracing on traction of the common carotid. But Unna[11] described no detectable influence on the renal blood flow in a 30 to 50 mm. Hg fall in arterial pressure due to distention of one carotidsinus. In our study in which a high pressure was given on a carotid sinus, the renal vascular vessels appeared to have no change in spite of a marked fall in systemic arterial pressure.

Stimulation of the central end of the cut femoral or sciatic nerve generally produced more or less renal vasoconstriction unrelated to the alteration of systemic blood pressure. This was in accordance with the result of our previous observation. But in 4 of the 42 experiments, a slight increase in renal blood flow was observed, which indicated vasodilatation of renal blood vessels. It is of interest that afferent impulses from these nerves could, unexpectedly, produce vasodilatation in the kidney, although it was elicited in merely exceptional cases.

On stimulation of the proximal end of the divided cervical vagal nerve, alteration in systemic blood pressure and responses in renal blood vessels were various. The renal vessels responded with no significant change in many experiments but occasionally either vasoconstriction or vasodilatation was observed. The hypotensive response accompanied renal vasodilatation more frequently. In 3 dogs, vasodilatation occurred with hypotensive response in low voltage stimulation and vasoconstriction with hypertensive response in high voltage. But it is still not clear whether a correlation between the systemic arterial pressure and the attitude of renal vessels is present or not, because in hypertensive response the latter was as various in the present study as observed in our previous report.

In general, stimulating procedures in the present study produced fewer significant alterations in renal blood flow in comparison with the results obtained from our experiments reported previously. These findings implicate following possibilities: (1) the nervous reflexes actually have less influence on renal vascularity; (2) the resistance intervening in the perfusing circuit was large enough to interfere the recorded results; and (3) operative manupulations during arterial canulation were apt to make more damage on the nervous tissue surrounding the renal artery. Further improvement in experimental conditions will be required to confirm the above-postulated hypotheses.

Summary

The renal blood flow was measured by an electromagnetic flow meter in dogs with the innervated kidney in situ perfused by donor's blood. Following results were obtained:

(1) In electrical stimulation of the distal end of the divided splanchnic nerve, renal vasoconstriction was produced.

(2) On stimulation of the distal end of the divided cervical vagal nerve, slight renal vasoconstriction occurred.

(3) By occlusion of common carotid arteries, slight renal vasoconstriction was produced.

(4) Giving a high pressure on the carotid sinus caused no alteration of renal vascular response.

(5) On stimulation of the central end of the divided sciatic or femoral nerve, the renal blood vessels showed generally vasoconstriction but rarely vasodilation.

(6) On stimulation of the central end of the divided cervical vagal nerve, the attitude of renal blood vessels was various.

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Studies on the Conduction System in Congenitial Malformations of the Heart, Especially of Tetralogy of Fallot

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For preventing complete AV block occasionally encountered during intracardiac operation, surgeons should have accurate knowledges of the anatomic course of conduction system in congenital malformations of the heart. The author studied on the conduction pathway of various malformed hearts, especially of tetralogy of Fallot by serial sections. After the investigation, the author noticed that in almost all cases of tetralogy of Fallot, the bundle of His was found on the left side of the ventricular septum around the apex. In the case of ventricular septal defect and of persistent common atrioventricular canal, the location of bundle of His was not invariable. From these observations, the author discussed on the surgical technique of radical repair of these anomalies.

SINCE Lewis[1] reported the successful closure of atrial septal defect under direct vision with the aid of general hypothermia in 1953, and Kirklin[2] and Lillehei[3] accomplished the complete repair of various ventricular septal defects using the pump-oxygenator in 1955, open heart surgery has made a rapid progress in accordance with the improvement of technique in general hypothermia and especially in extracorporeal circulation. As the number of patients who received intracardiac surgery was increased, some complications peculiar to this method attracted physician's attention. One of the most serious complications was complete heart block which was observed after this surgery.[4]-[10] According to the reports of Effler,[7] Allen[6] and Kirklin,[15],[16] this complete heart block showed high mortality rate, in spite of the introduction of Isuprel and electronic pacemaker.[11]-[14]

In our clinic, one of 12 cases of tetralogy of Fallot, 3 of 7 persistent common atrioventricular canal, and one of 47 ventricular septal defect died of complete AV block within 6 months after the operation. It is considered that anatomical confirmation of the conduction system is needed to prevent the developemet of AV block. Allen[17] reported a successful method of supravital staining of conduction system by iodine, but other authors[18] found the destruction of conduction tissue by iodine,

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and morever, it was difficult to obtain the completely bloodless field necessary for the supravital staining.[19)-21)] Since we do not have an adequate method to visualize the conduction system during operation, we must study and know its principal anatomic course beforehand. Some investigators[22)-25)] discussed the course of this conduction system in their recent papers. This paper is to report the result of analysis based on quantitative study of hearts of ventricular septal defect, persistent common atrioventricular canal and above all tetralogy of Fallot, and to present a safer technique in the surgery of cardiac septal defect.

Materials and Methods

Ten specimens of tetralogy of Fallot, 2 of ventricular spetal defect, and 2 of persistent common atrioventricular canal were subjected to an intensive study. All of them were operated upon in our clinic or dissected at the Department of Pathology, University of Tokyo (Table I).

Table I. Anatomic Data on the Patients Studied

	Case	Disease	Age	Sex	Body length (cm.)	Body weight (Kg.)	Heart weight (Gm.)	Diameter of the ventricular septal defect (mm.)
1	Y. K.	Tetralogy of Fallot	4 Yrs. 5 Mon.	M	95.0	14.0	90.0	13.0
2	Y. O.		5 Yrs. 8 Mon.	F	101.0	16.0	120.0	15.0
3	H. S.		1 Yrs. 3 Mon.	M	71.0	6.7	45.0	10.0
4	K. A.		9 Yrs. 3 Mon.	M	153.0	19.0	130.0	15.0
5	T. Y.		14 Yrs. 6 Mon.	F	144.0	32.0	275.0	14.0
6	N. K.		6 Yrs. 4 Mon.	F	107.3	18.5	145.0	13.0
7	T. K.		19 Yrs. 2 Mon.	M	171.0	50.0	350.0	22.0
8	S. T.		6 Yrs. 11 Mon.	M	98.3	12.0	85.0	11.0
9	N. M.		3 Yrs. 10 Mon.	M	95.0	11.0	80.0	11.0
10	H. K.		10 Yrs. 2 Mon.	F	123.0	25.0	270.0	17.0
11	M. T.	Ventricular septal defect	3 Days	F	49.0	2.365	24.0	8.0
12	S. H.		25 Yrs. 5 Mon.	F	156.0	59.0	470.0	8.0
13	Ta. K.	Endocardial cushion defect	3 Yrs. 11 Mon.	M	100.0	14.0	140.0	19.0
14	Hi. K.		7 Yrs. 4 Mon.	M	111.0	17.0	135.0	24.0

All specimens were opened by conventional method, or occasionally by Oppenheimer's,[26)] and fixed in 10% formaldehyde solution. Inside of both ventricles were photographed precisely, then a block of tissue was removed by Lev's method,[27)] including the ostium of coronary sinus, the inferior portion of atrial walls and interatrial septum, the superior portion of ventricular septum, the anterior portion of moderator band, or mid-portion of pulmonary ostium in tetralogy of Fallot, and the attached fibrous ring of the heart. The block was

subdivided into 3 to 9 blocks in a direction corresponding approximately to the frontal plane of the heart. The AV node beneath the coronary sinus, bundle of His, and its bifurcation could be identified by naked eyes at careful sectioning. The right bundle branch (RBB) was also visualized at the moderator band. All blocks were embeded in paraffin, and tissue sectioning was done at 7μ thickness. Every 10th section was saved through all blocks, but occasionally complete continuity was obtained if necessary. They were stained with Masson trichrom, sometimes with Weigert-and-Van Gieson stains, Mallory-Heidenhain, PAS, or Hämatoxylin and eosin.

Results

(A) Macro- and Microscopic Findings of Tetralogy of Fallot:

Specimen #1: Y. K. 4 Yrs. 5 Mo. M.

The heart showed a severe infundibular and valvular pulmonary stenosis with 2 cuspid pulmonary valve, and interventricular septal defect 13 mm. in antero-posterior diameter.

Histologically, the AV node originated from the atrial muscle of dorsal portion of right atrium, located directly beneath the ostium of coronary sinus.

The AV bundle proceeded ventrally to the central fibrous body or fibrous trigone (CFB) accompanied by R. septi fibrosi. The AV bundle of 800μ in

Fig. 1. Conduction system in specimen #1. (Refer to method of sectioning in text). Arrow shows bifurcation into left and right bundle branches. R indicates right bundle branch; L, left bundle branch; D, interventricular septal defect. Masson trichrom; ×7

Fig. 2. Conduction system in specimen #2. Arrow shows bifurcation. Masson trichrom; ×7

thickness penetrated CFB, giving off numerous paraspecific communications[28)-31)] to the ventricular septum. Reaching the ventricular septum, a posterior portion of left bundle branch (LBB) was sent off, therefrom AV bundle came to ventricular septal defect, and right bundle branch (RBB) was given off at a point 17 mm. ventral from the posterior rim of the defect (Fig. 1).

Although the point was located at the apex of ventricular septum, the muscular mass of about 2 mm. in thickness was retained on the right side of the location.

While LBB ran down along the left side of ventricular septum spreading as that of a membrane beneath the endocardium, RBB penetrated into the septal musculature, and appeared to terminate there. This may be due to the difficulty in following up its further branching. Around the terminal portion of the RBB, many nerve fibers were found but had no close relation to the conduction tissue.

Specimen #2: Y. O. 5 Yrs. 8 Mo. F.

There was an infundibular and valvular pulmonary stenosis. The ventricular septal defect measured 15 mm. in diameter. Histologically, the location of the AV node and atrial portion of AV bundle were seen as in the previous case. After the AV bundle of 1.4 mm. in thickness passed through CFB, it deviated left ventricle and reached its subendocardial layer, where LBB was separated. While this point was 3.8 mm. distant from the top of septal ridge, the bundle of His went forward, reaching the defect, and gave off RBB at the point 0.76 mm. ventral to the posterior rim of the defect (Fig. 2). Though the remainder of LBB went down the ventricular septum along its subendocardium, RBB penetrated deeply into the septal musculature, encircled by dense firous tissue, where it became as thinner as 130 μ in thickness and reached the mid-sagittal plane of septum at a site 1.8 mm. ventral to the posterior rim of the defect, where it located at 1.6 mm. deep from the endocardium of lower margin of defect. Finally RBB was recognized in the subendocardium of the moderator band, expanding to 500 μ in thickness, and went to the anterior papillary muscle along it.

Specimen #3: H. S. 1 Yrs. 3 Mo. M.

The heart revealed the infundibular and valvular pulmonary stenosis as well as the ventricular septal defect, 10 mm. in diameter, and the large interatrial foramen. The histological findings of AV node and AV bundle were similar to those in the previous case. The AV bundle of 1.0 mm. in thickness deviated rapidly towards the subendocardial layer of left ventricle after its penetration through CFB, and gave off LBB. While the bifurcation was 2.8 mm. ventral to the posterior rim of the defect (Fig. 3), RBB reached the mid-sagittal plane at the point 5.5 mm. distal to the posterior

Fig. 3. Conduction system in specimen #3. Arrow shows bifurcation. Masson trichrom; ×7

Fig. 4. Conduction system in specimen #4. Arrow shows bifurcation. Masson trichrom; ×7

edge of the defect, lying at 2.0 mm. deep from the endocardium of septal summit.

Specimen #4: K. A. 9 Yrs. 3 Mo. M.

There were the infundibular and valvular pulmonary stenosis, the ventricular septal defect measuring 15. mm. in diameter, and a small atrial septal defect.

Histologically, the AV bundle of 1 mm. in thickness penetrated CFB, and deviated 2 mm. towards left ventricular side from the septal ridge, but its bifurcation located at 0.6 mm. dorsal to the posterior rim of the defect in this case (Fig. 4).

While the course of LBB was similar to that of other cases, RBB arrived at the mid-sagittal plane at a site 0.5 mm. ventral to that rim, lying immediately beneath the septal endocardium of 700 μ in thickness.

Specimen #5: T. Y. 14 Yrs. 6 Mo. F.

This heart demonstrated the severe infundibular and valvular pulmonary stenosis, the interventricular foramen measuring 14 mm. in diameter and a large atrial septal defect. Microscopically, the bifurcation of AV bundle was 0.99 mm. dorsal to the posterior edge of defect, and it was 6.3 mm. distant from the summit of ventricular septum (Fig. 5). RBB reached the mid-sagittal plane at a site 1.7 mm. distal to that edge, lying at 3.3 mm. in depth from the endocardial surface of septal apex.

Specimen #6: N. K. 6 Yrs. 4 Mo. F.

This heart had also the infundibular and valvular pulmonary stenosis, associated with the ventricular septal defect measuring 13 mm. in diameter. Histologically, after crossing through CFB, the AV bundle veered its course

Fig. 5. Conduction system in specimen #5. Arrow shows bifurcation. Masson trichrom; ×7

Fig. 6. Conduction system in specimen #5. Section shows one muscular bundle, and no left bundle branch is found. Masson trichrom; ×7

into the septal musculature at a site 0.17 mm. proximal to the posterior rim of defect and deviating 8.2 mm. towards left from septal summit, without giving off any LBB (Fig. 6). The common bundle was 440 μ in thickness, and reached the mid-sagittal plane at the posterior edge of defect, 6.8 mm. deep from the endocardial surface of septal ridge, encircled by a light amorphous space, which may be a bursa (Curran)[28] or a fatty

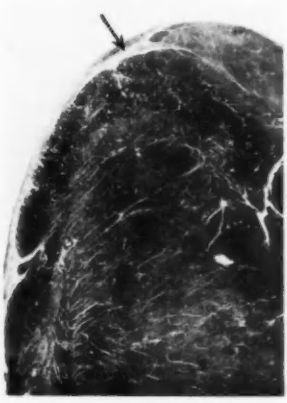


Fig. 7. Conduction system in specimen #7. Arrow shows bifurcation. Masson trichrom; ×7

Fig. 8. Conduction tissue in specimen #8. Arrow shows bifurcation. Masson trichrom; ×7

tissue (Mönckeberg).[32] Finally the muscular bundle came to the moderator band, but the structure like LBB could never be identified.

Specimen #7: T. K. 19 Yrs. 2 Mo. M.

This heart demonstrated the infundibular pulmonary stenosis and the ventricular septal defect measuring 22 mm. in diameter. Microscopically, the AV bundle deviated to left side after penetrating CFB, the bifurcating point of which was 3.7 mm. distal to the posterior rim of defect and 6.3 mm. distant towards left ventricle from septal apex (Fig. 7). The RBB reached the mid-sagittal plane at a site 5.6 mm. distal to the posterior rim of defect and 1.6 mm. deep from the endocardial surface of septal apex.

Specimen #8: S. T. 6 Yrs. 11 Mo. M.

There was pulmonary valvular stenosis associated with ventricular septal defect measuring 17 mm. in diameter. The AV bundle deviated to left ventricle, and its bifurcating point was 1.1 mm. distal to the posterior edge of defect (Fig. 8) and 0.7 mm. distant from septal ridge. While RBB reached the mid-sagittal plane at a site 1.6 mm. ventral to the posterior rim of defect, its further course was obscure in the septal tissue.

Specimen #9: N. M. 3 Yrs. 10 Mo. M.

The heart showed the infundibular and valvular pulmonary stenosis, as well as the interventricular foramen measuring 11 mm. in diameter. The bifurcating point of AV bundle was 1.3 mm. proximal to the posterior rim of defect (Fig. 9), and RBB reached the mid-sagittal plane at the

Fig. 9. Conduction system in specimen #9. Arrow shows bifurcation. Masson trichrom; ×7

Fig. 10. Conduction system in specimen #10. Arrow shows bifurcation. Masson trichrom; ×7

posterior rim of defect, 2.6 mm. deep from the endocardial surface of septal ridge.

Specimen #10: H. K. 10 Yrs. 2 Mo. F.

The heart had the infundibular and valvular pulmonary stenosis, and ventricular septal defect measuring 17 mm. in diameter. The AV bundle deviated toward left ventricular side following penetrating CFB, and bifurcated at a site 0.46 mm. proximal to the posterior edge of defect and 6.0 mm. distant to left side from septal apex (Fig. 10). RBB took its course right anteriorly, but always subendocardially.

(B) Macro- and Microscopic Observations on Ventricular Septal Defect:

Specimen #11: M. T. 3 Day. F.

This anomaly belonged to "the defect in the posterior part of the anterior septum with normal position of the arterial trunk" by Lillehei (Rokitansky),[33] or to "the defects related to ventricular outflow tracts and involving region inferior to crista supraventricularis" by Kirklin,[34),35] and the defect was measuring 8×12 mm. in size. The AV bundle located immediately beneath the right ventricular endocardium after penetrating CFB (Fig. 11), then ran across the septal muscle from right to left passing

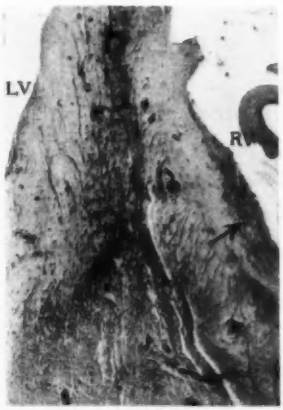


Fig. 11. Conduction system in specimen #11. AV bundle locates directly beneath the right ventricular endocardium. RV indicates right ventricle; LV, left ventricle. Masson trichrom; ×18

Fig. 12. Conduction tissue of specimen #11. Arrow shows bifurcation into right and left bundle branches. Masson trichrom; ×18

through the posterior rim of defect and appeared in the subendocardial layer of left ventricle. The site of bifurcation was at the middle point of antero-posterior diameter of the defect (Fig. 12), which was 1.5 mm. distant towards left side from the septal apex. The further course of both bundle branches were not unusual.

Specimen #12: S.H. 25 Yrs. 5 Mo. F.

The location of the defect of ventricular septum was the same as above case, the size of which was 8×5 mm. in diameters. Following the penetration through CFB, the AV bundle deviated to the left ventricular side, and the site of bifurcation was 0.2 mm. dorsal to the posterior rim of defect and 1.4 mm. distant towards left side from septal apex (Fig. 13). RBB came to the mid-sagittal plane at a point 0.2 mm. ventral to that rim, whereas it located at subendocardium.

Fig. 13. Conduction system in specimen # 12. Arrow shows bifurcation. In this section, septum is viewed from the front, therefore right side of this figure corresponds to left ventricle and left side to right ventricle. Masson trichrom; ×18

(C) Macro- and Microscopic Observations on Persistent Common Atrioventricular Canal:

Specimen #13: T. KU. 3 Yrs. 11 Mo. M.

This heart was considered as the complete form of endocardial cushion defect according to Wakai and Edwards,[36] associated with patent ostium secundum, hypoplasia of right ventricle, partial transposition of great vessels, as well as infundibular and valvular pulmonary stenosis with 2 cusps. The common atrioventricular orifice was measuring 19×22 mm. in diameters. Histologically, the AV node originated from the postero-inferior wall of right atrial musculature, being about 1 cm. remote from the rudimentary atrial septum, the relation of which to coronary sinus was not clear, since the specimen was damaged around the coronary sinus. The AV bundle passed through CFB to the interventricular septum, and initially located on the right ventricular side of the septum (Fig. 14).

Fig. 14. Conduction system in specimen #13. Septum is viewed from the front as in the previous case. Arrow shows the bifurcation. Masson trichrom; ×18

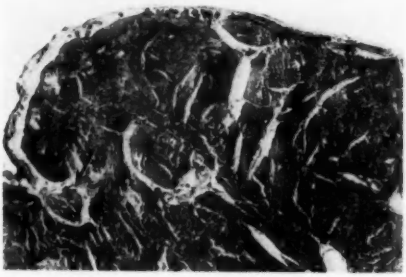


Fig. 15. Conduction system in specimen #13. Arrow shows AV bundle. Masson trichrom; ×18

Therefrom it ran across the septum from right to left around its summit, lying in 1.3 mm. deep from the endocardial surface of septal apex, giving off some fine muscular branches towards right side.

It appeared immediately beneath the endocardium of left-side ventricle at about a middle point of antero-posterior diameter of the orifice (Fig. 15). Thereafter it descended into the muscular prominence running down from the crista supraventricularis in the left-side ventricle.

Specimen #14: HI. K. 7 Yrs. 4 Mo. M.

This heart also belonged to the complete form of endocardial cushion defect, associated with the infundibular and valvular pulmonary stenosis with bicuspid cusps, as well as persistent left superior caval vein and coronary sinus which had their common opening in the left atrial cavity. The atrioventricular orifice was measuring 24×27 mm. in diameters. Microscopically, the AV node was identified in the dorsocaudal portion of right atrial musculature, whereas there were numerous nerve fibers or ganglionic cells around the coronary sinus. The AV bundle was found at the ridge of interventricular septum, after penetrating fibrous tissue (Fig. 16). Therefrom it deviated immediately towards left side, and divided into 2 branches at a site 3.9 mm. ventral to the posterior rim of the defect and 1.1 mm. distant towards left ventricle from septal ridge. While LBB streamed down along the septum as usual, RBB sent off some distinct branches into the septal tissue, as Lev[22] stated. RBB veered right inferiorly around the base of pulmonary artery (Fig. 17).

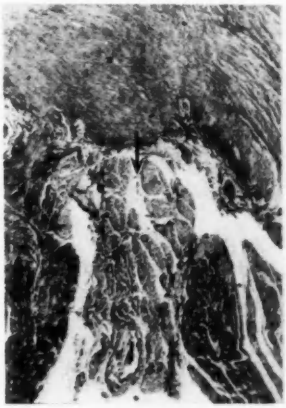


Fig. 16. Conduction system in specimen #14. AV bundle is shown at the ridge of septum. Masson trichrom; ×18

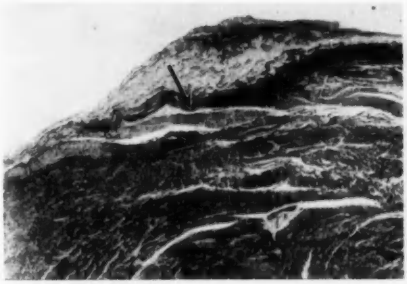


Fig. 17. Conduction system in specimen #14. Arrow shows right bundle branch. Masson trichrom; ×18

Discussion and Conclusion

The conduction system of the heart was described by His[37] and Kent[38] in 1893, by Tawara[39] in 1906, and by Keith and Flack[40] in 1907, and thereafter a great deal of literatures concerning the conduction system of the heart have been published. However, it has been a debate for a long time whether the conduction mechanism of cardiac impulse was myogenic[41] or neurogenic.[42]-[45] In case of complete heart block, Erlanger[46],[47] and Cullis[48] produced a complete heart block by the mechanical interruption of AV bundle, and Hoekenga,[49] Yater,[50] Lev,[51] described histopathological lesions of conduction tissue in the malformed hearts with congenital complete AV block. On the other hand, Hume[52] and Mahaim[53] reported complete AV block with slight or no pathological changes in conduction tissue. Hume[52] then suggested that the presence of functional heart block, and Rossi[45] assumed a neurogenic injury as the cause of the block. According to Etfler,[7] the complete AV block could be provoked more frequently by local ischemia in the septum or anoxia associated with poor perfusion rather than by mechanical injuries. Taufic[54] observed that the block did not occur even when the septum was interrupted from its blood supply, whereas Sirak[55] considered the edema or bleeding around the conduction tissue as an important factor.

Since Reemtsma[56] demonstrated histopathologically the presence of trauma or suture holes in the conduction bundles of patients who died of complete AV block during an intracardiac operation, it was generally suggested that the complete AV block was brought about by the direct

destruction of the conduction system.

The course of conduction tissue of ventricular septal defect was described by Mönckeberg,[32),57)] and that of persistent ostium primum by Mönckeberg[32),57)] and Morison.[58)]

The author wishes to summarize the findings of the present study and contrive some surgical techniques.

(A) Tetralogy of Fallot:

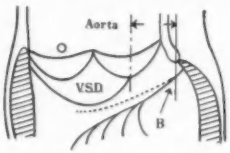
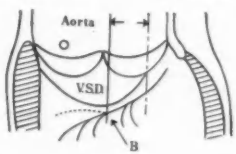
i) Portion from the AV node to the bifurcation of AV bundle:

The AV node located in the dorsal portion of right atrial musculature beneath the coronary sinus, and it transformed into AV bundle with no distinct boundary, accompanied by R. septi fibrosi. The paraspecific communications were found in most of all, which might be on account of their younger age. But such cases as Truex's,[25)] where the common bundle divided into its 2 branches above the fibrous ring, were not found in the present series. The common bundle appeared directly beneath the left ventricular subendocardium, immediately after penetrating CFB, in 9 of 10 cases.

ii) From the posterior rim of ventricular septal defect to the bifurcation (Table II):

The distance from the posterior rim of ventricular septal defect to the bifurcation is shown in Table II. Type I indicates the type in

Table II. Distance from the Bifurcation to the Posterior Rim of Ventricular Septal Defect

Type I		K. A.	(Proximal)	0.60 mm.	
		T. Y.	〃	0.99 mm.	
		N. K.	〃	0.17 mm.	
		N. M.	〃	1.3 mm.	
		H. K.	〃	0.46 mm.	
Type II		Y. K.	(Distal)	1.7 mm.	13%*
		Y. O.	〃	0.76 mm.	5%
		H. S.	〃	2.8 mm.	28%
		T. K.	〃	3.7 mm.	17%
		S. T.	〃	1.1 mm.	10%

Note: ← → indicates this distance; B, bifurcating point; V.S.D., ventricular septal defect; *, Ratio of this distance to the diameter of ventricular septal defect.

which bifurcation is posterior or proximal to the posterior rim of interventricular foramen, whereas Type II means the case where bifurcation is anterior or distal to it. They are schematically shown in Fig 18 and 19. Although there is no risk for complete AV block in the case of Type I,

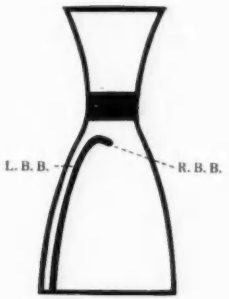


Fig. 18 (Left). Schema shows Type I of bifurcation into left and right bundle branches. Cardiac septum is viewed from behind. LBB indicates left bundle branch; RBB, right bundle branch. Note that the site of bifurcation is not related to the site of interventricular foramen.

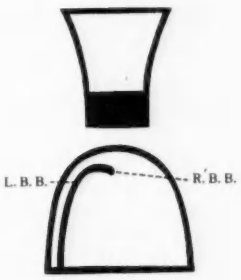
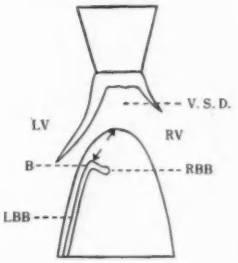


Fig. 19 (Right). Schema shows Type II of bifurcation into left and right bundle branches. Note that bifurcation locates at the lower margin of interventricular foramen.

it holds only 50%. In Type II, holding the remaining 50%, the bifurcating points are within proximal one third of antero-posterior diameter of the defect. Therefore, in closing the interventricular foramen, the care must be taken of that portion, which is usually called posteroinferior margin of ventricular septal defect. Moreover, Type I and Type II are not differentiated clinically.

iii) Distance from the septal ridge to the bifurcation (Table III):
It shows the deviation of the bifurcation towards left side from the

Table III. Distance from the Apex of Septum to the Bifurcation



Case	Distance
Y. K.	0 mm.
Y. O.	5.0 mm.
H. S.	2.0 mm.
K. A.	2.0 mm.
T. Y.	6.3 mm.
N. K.	8.2 mm.
T. K.	6.3 mm.
S. T.	0.7 mm.
N. M.	2.7 mm.
H. K.	6.0 mm.

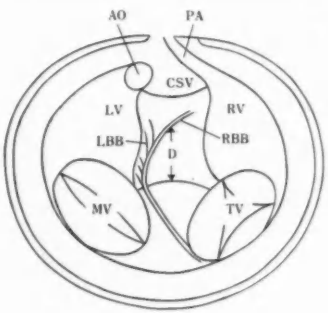
Note: ← → indicates distance; B, bifurcating point; LBB, left bundle branch; LV, left ventricle; RBB, right bundle branch; RV, right ventricle; VSD, ventricular septal defect.

ridge of the septum. It is clear that the method of utilizing the left ventricular side of septal apex for the repair must be abondoned. Contrariwise, the suture should be put on the right ventricular side of the septum.

iv) Course of RBB from the bifurcation to the moderator band (Table IV, V):

The running course of RBB in tetralogy of Fallot is generally similar

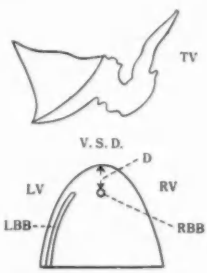
Table IV. Distance from the Posterior Rim of Ventricular Septal Defect to the Point Where RBB Reaches the Mid-Sagittal Plane of the Septum



Case	Distance (D)	Diameter of ventricular septal defect (d)	D/d
Y. K.	1.7 mm.	13 mm.	13%
Y. O.	1.8 mm.	15 mm.	12%
H. S.	5.5 mm.	10 mm.	55%
K. A.	0.5 mm.	15 mm.	33%
T. Y.	1.9 mm.	14 mm.	14%
N. K.	0 mm.	13 mm.	0%
T. K.	5.6 mm.	22 mm.	25%
S. T.	1.6 mm.	11 mm.	14%
N. M.	0 mm.	11 mm.	0%
H. K.	2.3 mm.	17 mm.	14%

Note: Ao, aorta; CSV, crista supraventricularis; LBB, left bundle branch; LV, left ventricle; MV, mitral valve; PA, pulmonary artery; RBB, right bundle branch; RV, right ventricle; TV, tricuspid valve.

Table V. Depth from the Endocardial Surface of the Septum to RBB at the Mid-Sagittal Plane of Septum



Case	Depth (D)
Y. K.	0 mm.
Y. O.	3.9 mm.
H. S.	2.0 mm.
K. A.	0.7 mm.
T. Y.	3.3 mm.
N. K.	6.8 mm.
T. K.	1.6 mm.
S. T.	1.6 mm.
N. M.	2.6 mm.
H. K.	0 mm.

Note: LBB; left bundle branch; LV; left ventricle; RBB; right bundle branch; RV, right ventricle; TV, tricuspid valve; VSD, ventricular septal defect.

to that in normal hearts. But in specimen #2 and #8, their ways disappeared shortly after they started. Those cases had the ECG consistent with tetralogy of Fallot, and their histologic findings were different from those in congenital absence of RBB stated by Coakley.[59] Embryologically, tetralogy of Fallot is derived from the failure of bulbar septum to close the interventricular formen completely. Mall[60] stated that the conduction tissue was the remnant of auricular canal on the one hand, Retzer,[61] Tandler,[62] and Walls[63] insisted upon the neomorphic development of AV bundle on the other, while Walls[63] claimed that RBB developed later than LBB. According to these opinions, disappearance of RBB in these cases might be due to abortive development of the original RBB. From Table IV, it can be decided that RBB reaches the mid-sagittal plane of the septum within one half of diameter of defect from its posterior rim. Since we know from Table V that RBB lies in zero to 6.8 mm. in depth from the endocardium of septal apex, it seems probable to interrupt it around the ridge, if suturing is deep enough.

V) Course of LBB after branching:

The both anterior and posterior portions stream down the left ventricular side of septum as usual, and their upper limits are always on the left side of it.

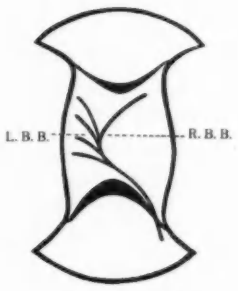
In specimen #6, LBB was not identified. The absence of LBB in a case of persistent ostium primum was reported by Morison.[58] Keith[64] stated that LBB was not a constant structure in any mammalian heart. In our case, the absence of LBB was not confirmed because ECG taken before operation was consistent with only severe right ventricular hypertrophy.

From the results mentioned above, the author considered that the corrective technique in tetralogy of Fallot should be modified. Lam[65] and Reemtsma[28] advocated the suture utilizing the left side of apex under defect, and Kirklin[67] introduced his method of the repair in which first step was to pass the needle from left to right through the ventricular septum at its junction with CFB and tricuspid ring. On the contrary, the author considered it was necessary to encompass the right ventricular side of the septum, as Lillehei[24] stated. Table II and III show that the needle must be pass through the right side of the septum when we suture the first one third of the diameter of the defect from the posterior rim of it, and thereafter through the septal ride (Table IV), in order to avoid any stenosis of outflow tract. Sirak[55] succeeded in prevention of surgical heart block by using a patch which was large enough to allow a 2 to 3 mm. overlap at the edge. This is considered to be due to the fact that the stiches are placed on the right ventricular side, when a patch which is larger than the real size of interventricular foramen is appleid by right ventriculotomy. Meanwhile, it is clearly understood from a review of Table IV and V that right bundle branch block (RBBB) could be readily

produced by closure of ventricular septal defect, as Lillehei[34] said, or by resection of infundibular stenosis. Zimmermann,[66] Kirklin,[15] and Hanson[68] reported the high incidence of RBBB in such cases. In our clinic, 15 out of 37 patients operated upon for ventricular septal defect developed complete RBBB postoperatively. Although most authors recognized no deleterious effect of RBBB, the question whether the direct injury of RBB has any influence upon cardiac function or not in such a case as #6, in which no LBB was identified, must be answered by further study.

(B) Ventricular Septal Defect:

The results of our study on the conduction tissue of ventricular septal defect are shown schematically in Fig. 20 and 21, which give the bird's-eye-



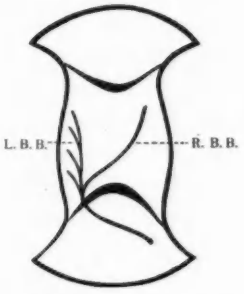


Fig. 20 (Left). Schema shows bird's-eye-view of ventricular septum of specimen #11. Upper part of this schema corresponds to anterior portion of the heart, and lower part to posterior portion. LBB indicates left bundle branch; RBB, right bundle branch.

Fig. 21 (Right). Schema shows bird's-eye-view of ventricular septum of specimen #12. Attention to the explanation of Fig. 20.

view of the ventricular septum. At the posterior edge of defect, the AV bundle locates on the right ventricular side of septum in one case, and on the left in another case.

Moreover, the AV bundle bifurcates at the far anterior site of that edge in the former, whereas it divides at the posterior site of that rim in the latter.

The course of AV bundle which Truex[25] considered as the commonest type was not observed in our cases. The fact that the AV bundle locates on the right side of septum must be borne in mind, when direct closure of the defect is attempted.

(C) Persistent Common Atrioventricular Canal:

The incidence of complete heart block is high in the corrective pro-

cedure of endocardial cushion defect, as Lillehei[8] or Kirklin[69] stated. Fig. 22 indicates that the AV bundle locates on the right ventricular side

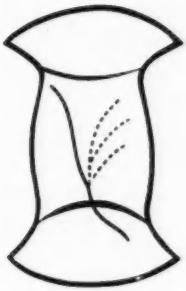


Fig. 22 (Left). Schema shows the same view as in Fig. 20 of ventricular septum of specimen #13.

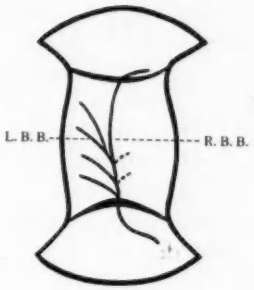


Fig. 23 (Right). Schema shows the same view as in Fig. 20 of ventriculhr septum of specimen #14.

of septum following its penetration from the fibrous tissue, that it veers to the left side of the septum with fine branches branching towards right side, and that it enters into the left-sided ventricle. Referring to some literatures, Ashoff[70] reported the transposition of the bundle branches, Morison[58] described the congenital abortive left bundle branch, and Keith[64] stated that LBB was not a contant structure in mammalian heart. In this case, a compact muscular branch entered into the ventricle of left side (therefore this ventricle should be called right branch ventricle, as Ashoff[70] said), and so-called LBB appeared abortive. The ECG in this case displayed only severe right ventricular hypertrophy. In the second case, as shown in Fig. 23, the AV bundle was recognized at the top of the ventricular septum, and gradually went towards left anterior, subsequently several right branches were given off, as Lev[22] described. In the persistent common atrioventricular canal, we must notice that the ridge of ventricular septum is relatively thin, which is partly due to the defective fusion of endocardial cushion, as Gunn and Dieckmann,[71] Moragues,[72] or Rogers[73] said. Therefore, in the intracardiac procedure, it is very important that sutures placed in the bare area of ventricular septum must not include large amount of tissue, as Kirklin[74] reported, to say nothing of utilizing the right ventricular side of the septum.

Summary

In an attempt to prevent complete AV block in the surgical repair of interventricular foramen, the course of specific conduction tissue was studied in 10 specimens of tetralogy of Fallot, in comparison with that in 2 cases

of ventricular septal defect and 2 of persistent common atrioventricular canal. The following results were obtained:

(1) Dislocation of AV node is not found in tetralogy of Fallot and ventricular septal defect, whereas the AV node is displaced a little bit posteriorly in persistent common atrioventricular canal.

(2) After passing through the central fibrous tissue, the AV bundle is recognized on the left of lower margin of the defect in 9 out of 10 specimens of tetralogy of Fallot, while it locates on the either right or left side in 2 specimens of ventricular septal defect, and on the right side or the top in 2 specimens of persistent common atrioventricular canal.

(3) In tetralogy of Fallot, the bifurcation is classified into 2 types. Each type includes the same number of cases. Differentiation of these 2 types by ECG is impossible.

(4) The LBB is not recognized in one case of tetralogy of Fallot, and of persistent common atrioventricular canal.

(5) In 2 cases of tetralogy of Fallot, the RBB is not clearly demonstrated.

(6) In surgical closure of the interventricular foramen, the stiches should be placed on the right ventricular side of lower margin of the defect at the proximal one third of the diameter.

(7) In closure of the anterior one half of the foramen, sutures should be placed on the ridge of the defect itself.

Acknowledgement

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An Analytical Study on Aberrant Ventricular Conduction

Part I. A General Rule

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and Kazuzo KATO, M. D.

In order to clarify the mechanism of aberrant ventricular conduction, the quantitative relationship between the ventricular cycle of a supraventricular impulse and the preceding ventricular cycle was analyzed graphically on 15 cases with supraventricular arrhythmia. A fairly sharp boundary curve, resembling the standard QT curve, between the conditions causing aberrantly and normally conducted beats, was obtained in each case. This result shows that the main factor of aberrant ventricular conduction is the refractory period of the intraventricular conduction system.

IT is well known that the aberrant ventricular conduction frequently occurs in the various types of supraventricular arrhythmia, when a short ventricular cycle follows a relatively long one. Since this phenomenon was first reported by Lewis[1] as aberrant ventricular response, many reports have been presented concerning its clinical significance and mechanism.

Though Scherf,[2] Gouaux and Ashman,[3] and Langendorf and Pick[4),5] showed as its mechanism that the refractory period following a long ventricular cycle is too long for the following supraventricular impulse with a short cycle to conduct the ventricle normally. Since no study on the quantitative correlation between the ventricular cycle of supraventricular impulse and the preceding cycle has been reported on clinical materials, the present study was undertaken to clarify the problems.

Materials and Method

Twelve cases of supraventricular premature beats, 2 cases of atrial fibrillation and one case of interference dissociation between sinus bradyarrhythmia

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and nodal escaped rhythm, whose electrocardiograms showed many aberrantly and normally conducted beats, were analyzed. In 2 cases of atrial fibrillation, digilalis was not given and only one type of bizarre ventricular complex, with complete and/or incomplete right bundle branch block pattern was shown. All these ventricular complexes could be differentiated from ventricular premature beats by the criteria by Langendorf and Pick.[5]

Every ventricular complex was plotted as one point in a diagram, the ordinate of which represents a ventricular cycle of the supraventricular impulse following a long cycle, and the abscissa of which represents the preceding long cycle. By this diagram, it can be examined whether a boundary condition of aberrant ventricular conduction may be found or not in each ventricular cycle.

Results

Case 1. 65-year-old man with an old myocardial infarction. The ECG (Fig. 1a) shows multiple supraventricular premature beats with varied

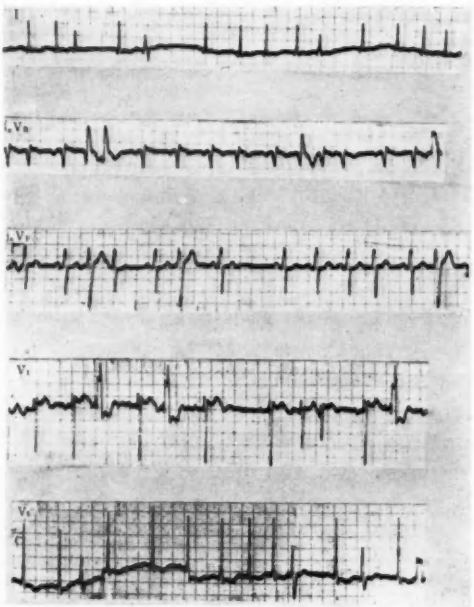

Fig. 1a.

coupling and preceding interval, and many aberrantly and normally conducted beats. Fig. 1b is the analytical diagram of this case, where the signals of small circle, x and dot represent normally, less aberrantly and aberrantly conducted beats, respectively.

In this figure, the area of the aberrant ventricular conduction is under that of normal conduction as a whole, and a boundary line showing upper limit of the condition causing aberrant conduction can be drawn fairly sharply, with some exceptions. This curve is alike an exponential curve, such as the standard QT curve referring to each ventricular cycle.

Case 2. 48-year-old man with hypertension and cerebral hemorrhage. The ECG (Fig. 2a) shows multiple atrial premature beats, and 2 types

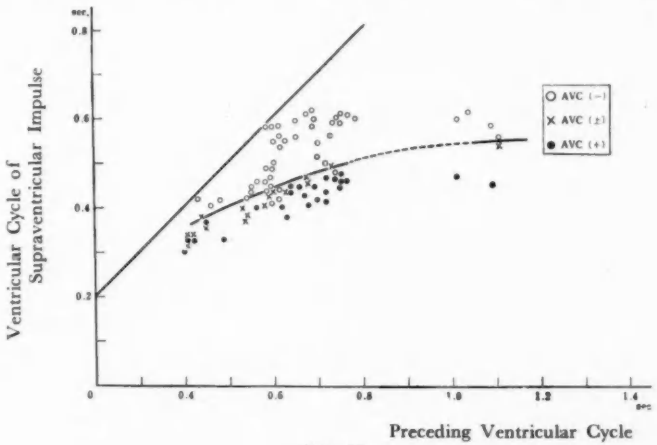

Fig. 1b.

of aberrantly conducted ventricular complex, that is, both right and left bundle branch block pattern. As seen in Fig. 2b, the boundary line is fairly sharp when the ventricular preceding cycle is long, but it is not the case when the preceding ventricular cycle is short especially when these beats appeared in succession.

Case 3. 35-year-old man with mitral valvular disease. The ECG (Fig. 3a) shows atrial fibrillation, aberrantly conducted beats with com-

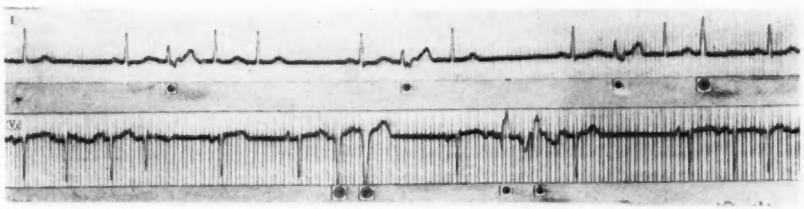

Fig. 2a.

plete and incomplete right bundle branch block pattern, and short runs of the bizarre ventricular complexes. Fig. 3b represents the analysis of

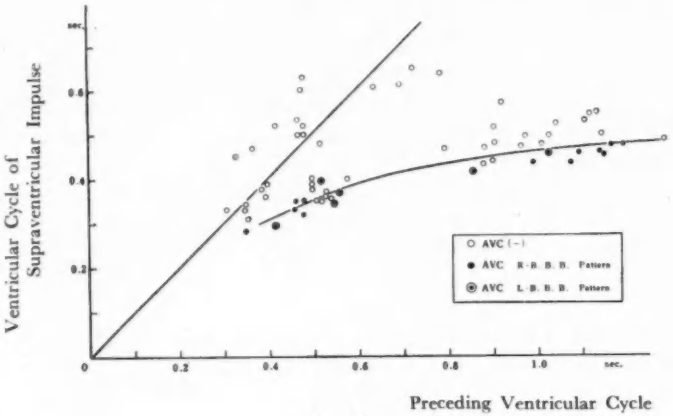

Fig. 2b.

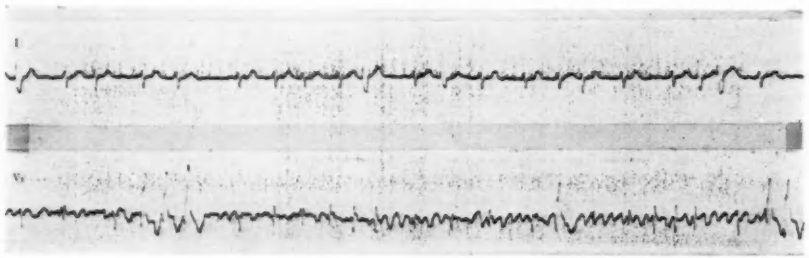

Fig. 3a.

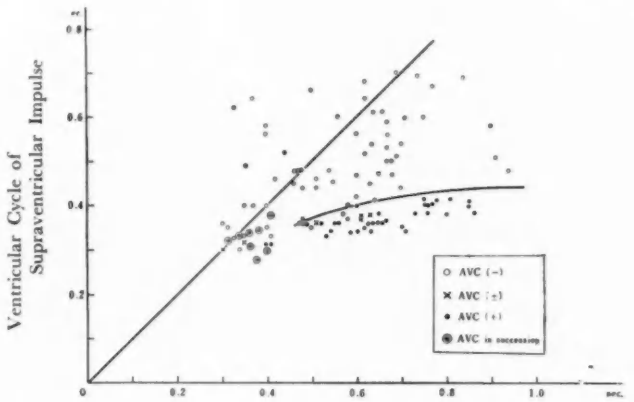

Fig. 3b.

the boundary condition, and shows almost the same findings as in case 2.

The analytical studies on other cases are almost the same, and a boundary line can be traced in a wide range of ventricular cycle in each case, if some irregularities are ignored. Fig. 4 represents the summary of

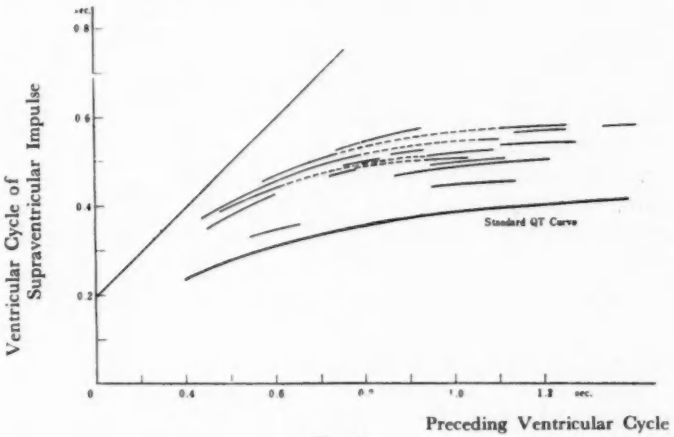

Fig. 4.

the analytical studies in all cases. As seen in this figure, the course of the boundary curves are essentially similar, though the level of this curve is somewhat different case by case. Furthermore, these curves occupy comparatively upper position above the standard QT curve in this analytical figure.

Discussion

Though the phenomenon of aberrant ventricular conduction is well known since about 40 years ago, its precise mechanism has not been sufficiently clarified. In 1925, Lewis and Master[6] demonstrated that the refractory period of atrioventricular conduction system was determined almost only by the length of the immediately preceding cycle, and Mendez and associates[7] clarified the similar relationship on the ventricular refractory period. According to these experimental studies, more than one preceding ventricular cycles, regular or irregular, have almost no cumulative effect on the refractory period to be questioned.

This is the theoretical background on which the present study was performed.

Fig. 4 shows clearly that the condition in which aberrant conduction occurs, is mostly determined by the relation between the preceding cycle and the following cycle and that the boundary line is represented as a

limited curve resembling the standard QT curve. This may be the clinical representation concerning with the refractory period of ventricular conduction system, and may be understood analogously to the experimental studies concerning the refractory period of atrioventricular conduction system by Lewis and Master and that of ventricular muscle by Mendez and associates.

A few factors, which may modify the boundary condition in this diagram must be considered here. The first is the fact that the ventricular cycle of the premature supraventricular impulse can not always represent the true time interval between the 2 ventricular excitations, but it may contain the silent intraventricular conduction time. This phenomenon was demonstrated in the cases represented by Burchell[8] and Holzmann,[9] whose ECGs show supraventricular arrhythmias with 2 types of aberrantly conducted beats, left and right bundle branch block pattern. This silent intraventricular conduction time must be considered to disturb the sharp delineation of boundary conditions in some cases. This factor will be discussed in the following paper.

The second factor is concerning with the irregularity of boundary conditions frequently seen in the short preceding cycle especially in the succession of aberrantly conducted beats. This phenomenon is well explained by Gouaux and Ashman at least in some parts. On this point, there remains some uncertain problems that will be discussed in the following paper.

The third problem is the difference of the level of the boundary line in each case. The presence or severity of some pathological process in the ventricular conduction system[10),11)] may be one factor for this problem, but the individual difference of refractoriness may play a role. The number of cases in this study is too small to analyse these factors. It is worth to be mentioned that the level of boundary lines is distinctly high above the standard QT curve in all cases. This shows that the refractory period of the ventricular conduction system is longer than that of the ventricular muscle.

Though these problems remain unsettled, a general rule of the quantitative relationship between preceding ventricular cycle and following cycle in the phenomenon of aberrant ventricular conduction is fairly clearly demonstrated in this clinical study, and this analytical method may contribute to the differentiation of ventricular premature beat from aberrant ventricular conduction in some cases of atrial fibrillation.

Summary

(1) In 15 cases of supraventricular arrhythmia with many aber-

rantly conducted beats, a relationship between the ventricular cycle of a supraventricular impulse and the preceding ventricular cycle was analysed, and the fairly limited boundary line concerning aberrant ventricular conduction was able to be traced in an analytical diagram in every case.

(2) Though the level of this boundary curve was somewhat varied in each case, its position in the analytical diagram was higher than the standard QT curve, and its figure was similar to the standard QT curve as a whole in every case.

(3) These results clearly showed that the main factor for aberrant ventricular conduction is the length of refractory period of the intraventricular conduction system following the preceding ventricular cycle.

(4) Several factors which might modify such a relationship were briefly discussed.

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Case Report

A Case of Congenital Aneurysm of the Pulmonary Artery

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and Shigeo OKINAKA, M. D.

ANEURYSM which arises from the truncus of the pulmonary artery is rare. Until 1950, a total of 152 cases of this disease have been reported.[14] Since the first confirmed case of the aneurysm of the pulmonary artery in Japan reported by Yoshida in 1934,[11] 18 cases have been reported, of which 11 cases were confirmed at surgery or autopsy. In this report, a case with a congenital aneurysm of the truncus of the left pulmonary artery with lethal outcome due to an acute exacerbation of the chronic pancreatitis and heart failure is presented.

Case Reports

A 43-year-old male, office worker, entered to Tokyo Univ. Hospital on January 18th, 1960, with chief complaints of dyspnea and epigastric pain. In his past history, a deformity of the chest (the prominence of the left chest wall with a marked pulsation) was noted by his parents at the time of his birth. The patient was not able to walk until 5 years of age, when a diagnosis of congenital heart disease was made for the first time by the family physician. Except the intolerance to strong physical exertion, his growth was normal. The deformity of the chest, however, became more apparent as he grew.

In 1945, when the patient was 29 years old, he visited our clinic for the first time with a complaint of the palpitation on slight exertion. Since that time, the episode of the attacks of palpitation and dyspnea occurred several times, repeating the remissions and exacerbations. For about 3 years prior to the present admission, nocturnal attacks of severe dyspnea occurred repeatedly, particularly in winter. In the beginning of December, 1959, when the patient caught a cold with the symptoms of cough and fever, the attacks of dyspnea became more marked. Prior to the admission, severe abdominal pain, nausea, vomiting and generalized exanthema of the skin were noted. The patient himself recognized a tender epigastric mass of the chicken egg size.

Physical examination on admission revealed a well developed, rather slender male with poor nutrition. The temperature was 38.6°C, pulse was feeble. The

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consciousness was clear, and the face was edematous. Slight cyanosis was noted in lips and tips of the fingers. The entire skin was covered with generalized exanthema which was diagnosed as toxicoderma due to phenobarbital. There was no evidence of jaundice. There was a slight clubbed fingers. The tongue was coated with a thick whitish material. A prominence of the left wall of the chest with a visible pulsation was noticed. The rhythm of the pulsation was paradoxical to the apical beat indicating the pulsation of the adjacent artery. The blood pressure was 108/58 on the right and 106/52 on the left. The left border of the cardiac dullness extended to the left anterior axillary line, while the upper and right border were not markedly shifted. A grade III systolic murmurs with a click was heard over the precordium with its maximum at the 2nd to 3rd intercostal space and at the left parasternal line. No diastolic murmurs were heard. A slight systolic thrill was felt at the 2nd intercostal space on the left (Fig. 1).

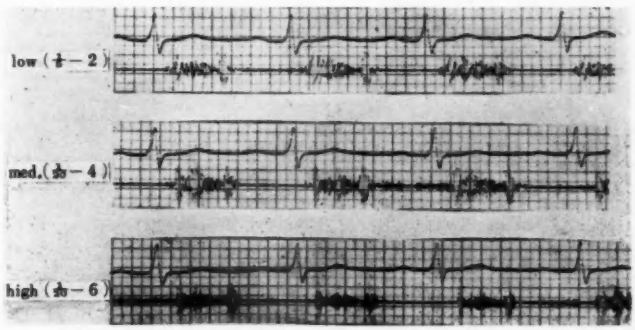

Fig. 1. Phonocardiogram at the pulmonary area.

In the lung field, moist rales were heard at the base posteriorly. In the epigastrium, a mass of the chicken egg size with a firmness suggesting a malignancy was palpated. Liver was palpable about 4 finger breadth below the right costal margin. Spleen was not palpable. No abnormalities was found on neurological examination. Slight edema was observed on the both legs.

Laboratory Findings: R. B. C. 559×10^4, Hb. 104%, W. B. C. 13,900 with slight increase in the band formed neutrophils. Urine; protein (+), sugar (−), no abnormal findings in the urinary sediment. Stool; positive occult blood. Blood sedimentation rate; 10 mm./hour. Chest X-ray (Fig. 2, 3, 4) showed a clearly defined mass in the left lung field extending from the mediastinum to the upper left field. On fluoroscopic examination, the tumor had extended from the mediastinum to the anterior upper part of the left lung. The border of the tumor was clearly defined, showing a marked pulsation with a phase just reversed to the contraction of the left ventricular wall demonstrating that the pulsative tumor arose from an artery adjacent to the heart. Electrocardiogram showed a few ventricular premature beats and incomplete right bundle branch block, suggesting hypertrophy of the right ventricle (Fig. 5). Venous pressure at the

right cubital vein was 110 mm. H_2O. Upper GI series showed that the mass at the epigastrium was not related to the gastrointestinal tract. Liver function tests showed a 12.5% retention of Bromsulfalein 30 minutes after the injection (normal 0-5%) and zinc sulfate 11.8, indicating a slight disturbance of liver function. Icteric index of the serum was slightly elevated (15). Serum sodium, potassium and chloride levels were within normal limit. Serum amylase activity was reduced to 4 units (normal 8-16). Plasma total protein was 6.4 Gm/100 ml. with an increase in the gamma globulin; 46% (normal 16%).

Clinical course during the hospitalization: The patient was treated with chemotherapy and digitalis. The fever and exanthema gradually subsided. On the 7th day of admission, the body temperature became normal and exanthema

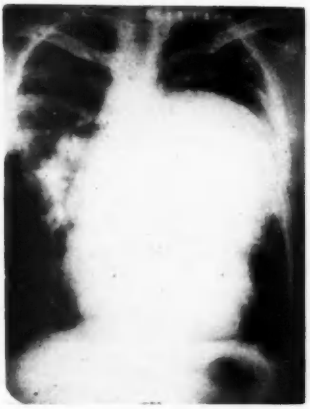

Fig. 2. A-P view.

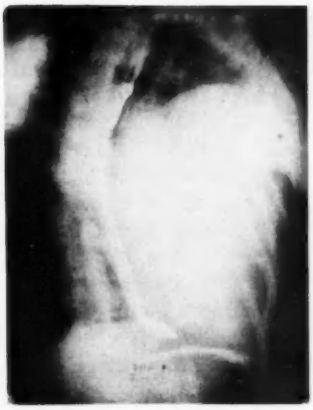

Fig. 3. First oblique view.

Fig. 4. Second oblique view.

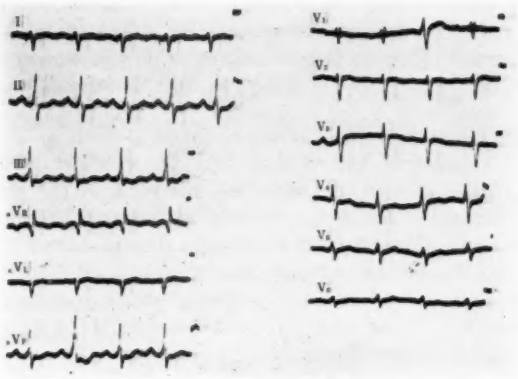

Fig. 5. Electrocardiogram

were substituted with desquamation. Moist rales in the lung also disappeared. Edema of the face and feet as well as hepatomegaly disappeared gradually. On February 10th, the liver was barely palpable at the epigastrium and the epigastric mass was hardly palpable and was not tender.

In spite of the clinical improvement, the patient began to complain of severe malaise, and thirst and polyuria became noticeable. The urine became strongly positive for glucose and fasting blood sugar was 268 mg./100 ml. Glucose tolerance test showed a typical pattern of diabetes mellitus. The injection of NPH insulin was began from February 17th. On the second day of the insulin injection, however, the patient developed an attack of hypoglycemia. This unexpected effect of the insulin injection on lowering the blood sugar level suggested a decreased secretion of insulin from the pancreas as the cause of hyperglycemia in this case. From the transitory increase in serum icteric index, reduced serum amylase level, and the hyperglycemia which was highly sensitive to insulin injection, it was suggested that the mass at the epigastrium was caused by an acute exacerbation of chronic pancreatitis, though there was no previous history suggesting the disease of the pancreas. From the 28th day of admission, the body temperature began to rise. Signs of bronchopneumonia appeared in both lung fields. On February 19th, the patient died of severe pulmonary edema. Because of the critical conditions of the patient during his admission, no cardiac catheterization was performed. From the fluoroscopic findings of the mass in the lung together with other clinical findings, a diagnosis of congenital aneurysm of the pulmonary artery was made antemortem.

Autopsy findings: The anterior upper portion of the left chest was occupied by an aneurysm of 10×8 cm in size. This aneurysm arose from the proximal part of the left pulmonary artery. The outer surface of the aneurysm was smooth and firmly adhered with pericardium. The wall of the aneurysm was rather thin and blood clots were found inside. The hypertrophy of the right ventricle was apparent and ventricular septal defect of about 1.5 cm. diameter was found at the upper portion of the septum. The valve of the left pulmonary

artery was bicuspid without significant stenosis. No luetic changes were observed in the walls of the aorta and pulmonary arteries. In both lung fields, changes due to bronchopneumonia were present. Congestion was seen in the liver, spleen, and kidneys. Consistency of the pancreas was increased, without apparent macroscopic changes.

Histological findings: Fig. 6 shows the histological findings of the wall of the aneurysm. As is shown in this picture, the elastic fibers of the walls of the aneurysm were torn demonstrating that this dilatation of the pulmonary artery was not a mere dilatation but an aneurysmal changes. The histological examination of the pancreas showed marked changes due to chronic pancreatitis. As is demonstrated in Fig. 7, infiltration with mononuclear cells was apparent. The normal structure of the pancreas was destroyed and scarcely any normal islet was left. The absence of the islet corresponded with the hyperglycemia which appeared immediately before death.

Fig. 6. The wall of the aneurysm.

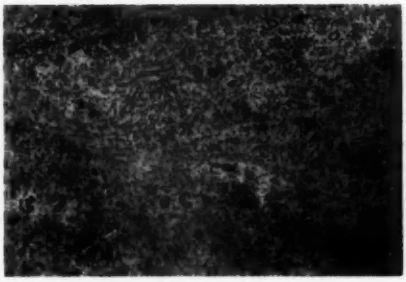

Fig. 7. Pancreas.

Discussions

Deterling and Clagett[15] reported the etiologic factors which cause the aneurysm of the pulmonary artery as follows: 1) syphilis, 2) congenital defect of the heart and blood vessels—patent ductus arteriosus, ventricular septal defect, atrial septal defect, hypoplasia, bicuspid pulmonary valve, double branch of subclavian artery—, 3) mycotic degeneration of the wall of the pulmonary artery, 4) atheroma, and 5) trauma. According to the report of Reid and Stevenson,[13] 47% of the aneurysma of the pulmonary artery has a congenital defect of the heart. Ventricular septal defect, atrial septal defect, and patent ductus arteriosus usually cause hypertension in the pulmonary artery resulting in the dilatation of the pulmonary artery. It is, therefore, suggested that a defect in the structure in the walls of the pulmonary artery might play an important role in the development of the aneurysm as well as the pulmonary hypertension. In the present case, a large ventricular septal defect and bicuspid pulmonary artery valve were demonstrated at autopsy.

From the history of the recognition of the deformity of the chest wall at birth, it would be reasonable to assume that the aneurysmal changes of the pulmonary artery in this patient was congenital in origin. In this case bicuspid pulmonary valve might cause slight pulmonary valvular stenosis, but a significant stenosis of the pulmonary valve was not found at autopsy. Long exsisting ventricular septal defect probably caused the hypertension of the pulmonary artery, which in turn was responsible for the dilatation of the congenitally weak left pulmonary artery, causing aneurysmal dilatation. On histological examination of the small arteries and arterioles of the lung, hyperplasia of the intima, and hypertrophy of the media of these vessels were found (Fig. 8), suggesting hypertension of the pulmonary artery.

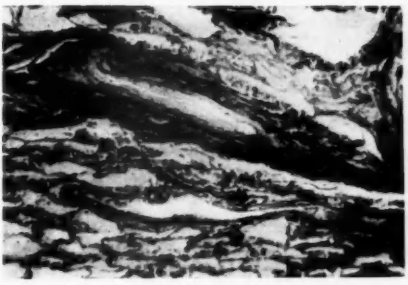

Fig. 8. Pulmonary arteriole.

Until 1950, 152 cases of aneurysm of the pulmonary artery were reported. In Japan, 18 cases of this disease have been reported. In Table I, all the reported cases of aneurysm of the pulmonary artery were summarized. Of these 18 cases, 10 were confirmed at autopsy and one was surgically confirmed. Among 18 cases, 7 were female, 5 male and sex was not described in the remaining 6. This almost equal incidence of the aneurysm in both sexes, as pointed out by Deterling and et al.,[15] is characteristic of the pulmonary artery aneurysm in contrast with the predominance of the males in the aneurysm of the aorta. In 11 autopsied and operated cases, congenital deformity of the heart was recognized in 6 cases—atrial septal defect in 5, and patent ductus arteriosus in one. Hypertrophy of the right ventricle was reported in 3 cases. The localization of the aneurysm was as follows—left pulmonary artery in one, right pulmonary artery in one, trunk of the pulmonary artery in 2.

Table I. Report of the Aneurysm of the Pulmonary Artery in Japan

No.	Reporter	Age	Sex	Autopsy	Etiology or complicated lesion	Location of the lesion	Wa-R.
1	T. Yo, et al.[1]	31	female	(−)	s. o. Eisenmenger complex		(−)
2	N. Muta, et al.	48	male	(−)	s. o. atrial septal defect	main pulmonary artery	
3	Y. Miyazaki, et al.[3]	38	female	(+)*	atrial septal defect		
4	T. Tomomatsu, et al.[4]			(+)	atrial septal defect		
5	〃			(−)	s. o. atrial septal defect		
6	〃			(−)	s. o. stenosis of pulmonary artery		
7	〃			(−)	s. o. stenosis of pulmonary artery		
8	〃			(−)	s. o. atrial septal defect		
9	S. Isoda, et al.[5]	4	female	Operated	s. o. ventricular septal defect		
10	〃	7	female	(+)	atrial septal defect		
11	H. Nozaki, et al.[6]	52	male	(+)		bilateral pulmonary artery	(−)
12	〃	19	male	(−)	s. o. patent ductus arteriosus		
13	N. Ayuzawa, et al.[7]	9	female	(+)*		main pulmonary artery	
14	K. Takahashi, et al.[1]	33	female	(+)		left pulmonary artery	
15	T. Maki, et al.[9]	34	male	(+)*	atrial septal defect		
16	S. Koide, et al.[10]	37	female	(+)	patent ductus arteriosus	main pulmonary artery	
17	M. Yoshida[11]	58	male	(+)			
18	T. Fukai[12]			(+)			

* Hypertrophy of the right ventricle

Summary

An autopsied case with a congenital aneurysm of the pulmonary trunk accompanied with ventricular septal defect and bicuspid pulmonary valve was reported. The literature of the pulmonary arterial aneurysm in Japan was briefly reviewed.

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14. Blades, B., Ford, W., and Clark, P.: Circulation **2**: 565, 1950.
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THE PHYSIOLOGICAL SOCIETY OF JAPAN

Program of 38th Annual Meeting

April 22–24, 1961
Kyoto Prefectural Medical University
Kyoto

(Only the topics relating to cardiology are selected.)

Respiration and Circulation (No. 18–30)

18. Tolerance to severe oxygen deficiency and its relation to the plasma potassium and magnesium levels. S. Kawashima and K. Kamakura (Nara Med. Coll.)
19. Chemical stimulation to respiration during the inhalation of hypoxic air in dogs. S. Hasumura, T. Natsui, and Y. Honda (Sch. of Med., Kanazawa Univ.)
20. Significance of carotid glomus in the secretion of epinephrine. T. Fukuda and K. Kobayashi (Chiba Univ., Sch. of Med.)
21. The distribution of fine vessels in human skin. Y. Ogawa, K. Yusa, and M. Takahashi (Yokohama Univ., Sch. of Med.)
22. Morphological investigations of blood circulation in human brain. G. Hiramitsu
23. Criticism and implication for method of hemodynamical research. Y. Inagaki et al. (Chiba Univ., Sch. of Med.)
24. Activity of cardio-regulatory nerve and abdominal sympathetic nerve in the Bainbridge reflex. I. Nishida, H. Okada, and K. Okamoto (Sch. of Med., Tottori Univ.)
25. Circulatory function in the sleep. K. Asahina, Y. Matsui, and S. Fujimoto (Sch. of Med., Toho Univ.)
26. Electrocardiographic studies on exertion with the telemeter. (1) Electrocardiogram in Marathon-runner. H. Matsui and K. Takagi (Nagoya Univ., Sch. of Med.)
27. Effects of plasmin system on the circulatory system. S. Oshiba, H. Watanabe, M. Maruyama, T. Minohara and S. Okamoto (Kobe Med. Coll.)
28. Attitudes of peripheral vessels reacting to adrenalin and noradrenalin. M. Asano, K. Yoshida, and Y. Tatai (National Institute of Public Health)

29. Studies on Tetrodotoxin. (1) Its vascular effects. K. Nishimaru (ABCC), T. Tani, and S. Nishimaru (Institute of Angiology)
30. On the peripheral circulation and regional oxygen pressure. R. Kusachi and T. Koizumi (Tokyo Women's Med. Coll.)
60. Basic studies of conditioned reflex in the vasomotor reflex. H. Harano, T. Obonai, and K. Sunohara (Tokyo Univ. of Education)
65. Studies on the intrathoracic pressure and circulatory function (1). K. Shiraishi (Mitsui-Kosei Hospital), T. Nagashima, K. Hirota, T. Asami, and H. Toyota (Fac. of Med., Univ. of Tokyo)

Muscle and End Plate (No. 85–88)

85. Intracellular potential during the fibrillation of skeletal and cardiac muscle. M. Goto, T. Tamai, R. Abe, and S. Yanaga (Fac. of Med., Kyushu Univ.)
86. On the abolition of action potential in the canine ventricular muscle. K. Matsuda and T. Hoshi (Fac. of Med., Univ. of Tokyo)
87. Action potential of the myocardium and lymph heart (Comparison of intracellular and superficial lead). G. Kawabata and M. Oki (Yamaguchi Med. Coll.)
88. Action potential of atrial muscle and its relation to the length of atrial muscle segment. K. Uchiyama, et al. (Sch. of Med., Nihon Univ.)
119. Effects of Frequency of stimulation on the cardiac nerve. G. Kato (Sch. of Med., Keio Univ.), H. Ito, K. Wada, and D. Nagai (Tokyo Dent. Coll.)

Symposium C: Excitability of Myocardium

1. A finding about responsiveness of oxygen electrode in myocardium. S. Yagi and G. Mikami (Iwate Med. Coll.)
2. Mechanism of excitation-contraction coupling in cardiac cells. K. Fujino, T. Yamaguchi, and K. Suzuki (Sapporo Univ. of Med.)
3. Problems in the automatic excitation of myocardium. K. Uchiyama, et al. (Sch. of Med., Nihon Univ.)
4. Actions of cardiac nerves to the myocardial excitability. K. Matsuda, T. Azuma, and T. Sugano (Fac. of Med., Univ. of Tokyo)
5. Relations of action potential contour of A-V node to the stimulation in microelectrode method. T. Sano, K. Tsuchihashi, T. Takigawa, and T. Shimamoto (Tokyo Med. and Dent. Univ.)
6. Components of excitation transmission potential in the intracellular potential of the heart. M. Goto, T. Tamai, R. Abe, and H. Kawada

(Fac. of Med., Kyushu Univ.)

7. Myocardial relaxation in the study of comparative physiology. H. Irisawa, J. Kobayashi, and A. Irisawa (Hiroshima Univ., Sch. of Med.)
8. Relation of electrical and mechanical phenomena in myocardium. Significance of similarity of integral and mechanical curves of intracellular action potential. M. Maekawa, K. Suekane, and Y. Konishi (Fac. of Med., Kyoto Univ.)

Symposium D: Physiology of Adrenocortical Electrolyte Hormone

1. Regulating factors for urinary excretion of sodium and potassium. T. Fukuda, K. Kobayashi, and T. Koyama (Chiba Univ., Sch. of Med.)
2. Steroids and their relation to the excretion of sodium and potassium in rats. K. Nakao, K. Hiraga, M. Inaba, M. Hirai, Y. Murayama, and S. Kanamoto (Tokyo Jikei-kai Sch. of Med.)
3. Pathologic physiology of water and electrolyte regulating hormone. K. Hirata (Sch. of Med., Keio Univ.)
4. Role of adrenocortical electrolyte hormone in the tubular transport mechanism of electrolyte. H. Abe (Osaka Univ. Med. Sch.)
5. Studies on the regulation of aldosterone secretion. K. Oshima et al. (Sch. of Med., Nihon Univ.)

THE JAPANESE CIRCULATION SOCIETY

Program of 21st Kanto District Meeting

May 6, 1961
School of Medicine, Nihon University
Tokyo

1. On the change of QT interval and Q-II time in the digitalis therapy. M. Namikawa, S. Mihashi, D. Ogawa, H. Matsuoka, M. Shiina, and Y. Kinoshita (Second Dept. of Int. Med., Chiba Univ., Sch. of Med.)
2. Mechanism of producing the right heart loading in the experimental

chronic cor pulmonale and its relation to QRS deflection. S. Miyamoto, G. Kakinuma, R. Koga, Y. Sezai, and S. Akiyama (Dept. of Surg., Sch. of Med., Nihon Univ.)

3. Studies on screening of cardiovascular diseases. (3) Five-years follow-up of electrocardiogram. T. Kobayashi, et al. (Dept. of Int. Med., Branch Hospital of Fac. of Med., Univ. of Tykyo)
4. Statistical analysis of cases showing abnormal electrocardiograms. K. Oshima, et al. (Dept. of Int. Med., Sch. of Med., Nihon Univ.)
5. Process of returning of atrial fibrillation to sinus rhythm. M. Horikawa, Y. Sano, S. Nomoto, Y. Sasano, and T. Wakatsuki (Second Dept. of Int. Med., Tokyo Med. Coll.)
6. Two cases of chronic exsudative pericarditis. S. Murakami, H. Yamauchi, and S. Kato (First Dept. of Int. Med., Juntendo Univ. Med. Sch.)
7. Effects of some coronary dilators on the myocardial metabolism. T. Kobayashi, et al. (Dept. of Int. Med., Branch Hospital of Fac. of Med., Univ., of Tokyo)
8. Effects of a new coronary dilator 7-(γ-Diisobutylamino-β-benzoyloxy-propyl)-theophyllin (TH_{24}) in angina pectoris. E. Kimura, S. Ushiyama, N. Kojima, O. Kunishima, and K. Satake (Dept. of Int. Med., Nippon Med. Coll.)
9. Congestive heart failure and coagulation factors. E. Kimura, et al. (Dept. of Int. Med., Nippon Med. Coll.)
10. An autopsy record of isolated myocarditis. K. Nakao, et al. (Dept. of Int. Med., Sch. of Med., Gumma Univ.)
11. A case of pulmonary artery thrombosis. D. Oshima, H. Miyashita, W. Hanaoka, and Y. Yoshitoshi (First Dept. of Int. Med., Fac. of Med., Univ. of Tokyo)
12. A case of collagen disease complicated with pulmonary hypertension. Y. Ohyama, et al. (Dept. of Int. Med., Niigata Univ., Sch. of Med.)
13. Studies on recording of intracardiac pressure during cardiac surgery (1) Ventricular septal defect with pulmonary hypertension. S. Furuta, K. Ito, D. Kure, and M. Saigusa (Dept. of Surg., Fac. of Med., Univ. of Tokyo)
14. A case of pulmonary hypertension with obscure origin. Z. Ishimi, et al. (Toranomon Hospital) and Y. Takeda, et al. (First Dept. of Int. Med., Sch. of Med., Toho Univ.)
15. Ballistocardiography in the pulmonary diseases. H. Sasamoto, et al. (Dept. of Int. Med., Sch. of Med., Keio Univ.)
16. A case of idiopathic pulmonary insufficiency. S. Ohta, M. Suzuki, H. Kaneko, Y. Sato, and A. Ishibiki (Mishuku Hospital)
17. Clinical studies on aortic arch syndome and similar diseases.

M. Ikeda, et al. (Third Dept. of Int. Med., Fac. of Med., Univ. of Tokyo)

18. A case of coarctation of the abdominal aorta. Y. Ohyama, et al. (Dept. of Int. Med., Niigata Univ., Sch. of Med.)
19. A case of unusual aortitis. M. Ohkuni and M. Iijima. (Dept. of Pediat., Fac. of Med., Univ. of Tokyo)
20. Two autopsied cases of dissecting aneurysm showing an interesting course. K. Yamakawa, K. Kitamura, S. Suzuki, H. Takamizawa, and S. Matoba (Dept. of Int. Med.) and T. Nagahama (Dept. of Surg., Juntendo Univ. Med. Sch.)
21. A case of partial transposition of the great vessels. M. Hashimoto, A. Mizuno, G. Sakauchi, K. Atsumi, R. Omoto, and M. Saigusa. (Dept. of Surg., Fac. of Med., Univ. of Tokyo)
22. Pathologic physiology in the condition of extreme hypothermia. S. Miyamoto, et al. (Dept. of Surg., Sch. of Med., Nihon Univ.)
23. Effects of some drugs on the experimental cerebral vascular lesions. T. Aizawa, Y. Goto, T. Hasegawa, G. Araki, H. Takizuka, and S. Kanda. (Dept. of Int. Med., Sch. of Med., Keio Univ.)
24. A case which showed accelerated vascular reflex. K. Ishikawa, et al. (Dept. of Surg., Fac. of Med., Univ. of Tokyo)
25. Several problems in management of hypertension. (4) K. Uraya, et al. (Dept. of Publ. Health, Chiba Univ., Sch. of Med.)
26. Studies on water diuresis: On the tissue fluid of adrenal medulla. R. Shiojima (Dept. of Obstet. and Gynaec., Yokohama Univ., Sch. of Med.)
27. A case of pyelonephritis. S. Tojo, K. Hirose, S. Tsuchiya, T. Taguchi and A. Matsugaki (Dept. of Int. Med., Chiba Univ., Sch. of Med.)
28. Unilateral renal hypertension. Y. Yokoyama, H. Kiyota, G. Watanabe, A. Mikami, and T. Morita (Second Dept. of Int. Med., Niigata Univ., Sch. of Med.)
29. Studies on the vascular lesion in experimental renal hypertension. (1) Effect of high arterial pressure on the cerebral vessels. J. Takeuchi, T. Takeda, E. Uchida, R. Okada, and F. Shintani (Second Dept. of Int. Med., Fac. of Med., Univ. of Tokyo)
30. Effects of long-term administration of Benzothiadiazine in hypertension. T. Ueda, et al. (Dept. of Int. Med., Tokyo Jikei-kai Sch. of Med.)
31. Clinical studies on rheumatic fever in adults. I. Mikata, et al. (Dept. of Int. Med., Sch. of Med., Keio Univ.)
32. An adult case of rheumatic fever which showed an unusual course. S. Uchida, T. Abe, M. Honma, K. Imura, K. Aizawa, and T. Hayashi (Dept. of Int. Med., Sch. of Med., Toho Univ.)

33. Studies on the glucoprotein in the arteriosclerotic diseases. K. Ariga, et al. (Dept. of Int. Med., Sch. of Med., Nihon Univ.)
34. Analysis of thrombotest in the anticoagulant therapy. S. Okinaka, S. Hosoda, F. Terasawa, J. Fujii, and M. Ikeda (Third Dept. of Int. Med., Fac. of Med., Univ. of Tokyo)
35. Changes of platelets, especially of adhesive platelets in Master's two-step test and the effects induced by monoamine oxidase inhibitors. T. Shimamoto, et al. (Tokyo Med. and Dent. Univ.)
36. Studies on prevention of thrombosis. Results of comparative experiment on effects of Nialamide with that of Phenindione. T. Shimamoto, T. Fujita, T. Ishioka, T. Matumura, and T. Sasaki (Tokyo Med. and Dent. Univ.)
37. Studies on the effects of the electrical stimulation of the peripheral cardiac nerves upon the cardiac impulse-formation and ventricular muscular excitability. H. Ueda, et al. (Second Dept. of Int. Med., Fac. of Med., Univ. of Tokyo)
38. Transseptal left heart catheterization: Preliminary report. H. Ueda, et al. (Second Dept. of Int. Med., Fac. of Med., Univ. of Tokyo)
39. On the so-called oculocardiac reflex. H. Kudo, N. Watanabe, M. Satoyoshi, K. Kato, and K. Kitamura (Juntendo Univ. Med. Sch.)

Lecture

Vascular system of cardiac septum. T. Takeuchi (Dept. of Path., Sch. of Med., Nihon Univ.)

THE JAPANESE COLLEGE OF ANGIOLOGY

Program of 2nd Annual Meeting

May 14, 1961
Mainichi Kokusai Salon of Nagoya City
Nagoya

1. Arteriography of feet in perforation type of leprosy. S. Kochi (National Suruga-Leprosorium)
2. A case of dermatomyoangitis. M. Kase, J. Ono, and N. Yoshimasu (Dept. of Int. Med., Kanto Teishin Hospital, Tokyo)
3. A case of sudden thrombosis or embolism of the right digits during an attack of an acute myocardial infarction. T. Kusui (2nd Int.

Clin., Wakayama Med. Sch., Wakayama)

4. On intrahepatic circulation in portal hypertension. R. Imamura, F. Ando, Y. Yokoyama, F. Suzuki, and S. Yamamoto (2nd Surg. Dept., Univ. of Nagoya, Sch. of Med., Nagoya)
5. Some problems of Buerger's disease. K. Kamiya and Y. Oomura (1st Surg. Dept., Univ. of Nagoya, Sch. of Med., Nagoya)
6. Histological studies on arterioles of various organs in Buerger's disease. H. Akiyama, K. Aso, R. Kitagawa, K. Hidai, and S. Hatano (2nd Surg. Dept., Univ. of Tokyo, Fac. of Med., Tokyo)
7. Acute stage of Buerger's disease. A pathohistologic study of 5 cases. K. Ishikawa and S. Kawase (1st. Surg. Dept., Univ. of Tokyo, Fac. of Med., Tokyo)
8. Adrenomedullectomy in Buerger's disease. S. Kuwabara, E. Takao, M. Kanemoto, and K. Higuchi (2nd Surg. Dept., Univ. of Tottori, Sch. of Med., Yonago)
9. Pulseless disease and atypical coarctation of the aorta. T. Sunada and K. Inada (1st Surg. Dept., Univ. of Okayama, Sch. of Med., Okayama)
10. Pathologic morphology of the pulseless disease (the 2nd report) with special reference to the intimal lesions of the arteries. T. Nasu and M. Mamiya (Dept. of Path., Univ. of Shinshu, Sch. of Med., Matsumoto)
11. Histopathological studies of experimental angitis on dogs with allylamine. T. Aizawa, et al. (Dept. of Int. Med., Keio Univ., Sch. of Med., Tokyo)
12. Demonstration of gamma globulin in vascular lesions of hypertensive arteritis and hypersensitivity arteritis. G. Ohta (Dept. of Med. Zoology, Univ. of Kanazawa, Sch. of Med., Kanazawa)
13. Studies on the so-called exsudative and productive endarteritis. G. Ooneda, et al. (Dept. of Path., Univ. of Gumma, Sch. of Med., Maebashi)
14. Clinical study on the effect of voluntary hyperventilation upon the systemic blood pressure of hypertensive persons (Part 1). C. Nagashima, T. Hiramatsu, and R. Hiramatsu (Coll. of General Education, Univ. of Tokyo, Tokyo)
15. On the blood circulation of the lower limb when the sciatic nerve and the spinal cord were injured. T. Ishizawa (Dept. of Orthoped., Kyoto Prefectural Univ., Med. Coll., Kyoto)
16. Polarographic studies on the peripheral circulation in the case of several nervous and vascular diseases. II. The responses to the body heating. A. Morisawa, Y. Yamane, H. Fukaya, S. Watanabe, K. Koide, and K. Seki (1st Dept. of Int. Med., Univ. of Tokyo, Fac. of Med., Tokyo)

17. Autonomic innervation of the inner ear vessels—an experimental study—Y. Nomura, M. Nagakura, S. Sugiura, and Y. Matsuba (Dept. of Oto., Univ. of Tokyo, Fac. of Med., Tokyo)
18. Studies on the microcirculation of nasal septum. T. Shiroiwa, I. Watanabe, H. Aoyagi, K. Mukai, and M. Tokashiki (Dept. of Oto., Tokyo Med. Coll., Tokyo)
19. Effect of carotid sinus reflex on renal circulation. T. Tomomatsu, et al. (1st Dept. of Int. Med., Kobe Med. Coll., Kobe)
20. Effect of adrenalmedullectomy on the supersensitivity of sympathectomized vessels. M. Oda (2nd Physiol. and Surg. Dept., Univ. of Tottori, Sch. of Med., Yonago)
21. Histochemical study on the autonomic innervation of the blood vessels. S. Okinaka, M. Yoshikawa, M. Uono, H. Tanabe, and M. Tomonaga (3rd Dept. of Int. Med., Univ. of Tokyo, Fac. of Med., Tokyo)

Special Lectures

1. Study on vasomotor tone of human digital vessels in the finger tips. K. Ishikawa (1st Surg. Dept., Univ. of Tokyo, Fac. of Med., Tokyo)
2. On the vascular sensory nerve. T. Kimura (Second Dept. of Surg., Univ. of Kyoto, Sch. of Med., Kyoto)
3. Plethysmographic study of blood vessels in various parts of the body surface. K. Takagi (Dept. of physiol., Univ. of Nagoya, Sch. of Med., Nagoya)
4. The autoregulation of the kidney circulation. C. Nagashima (Coll. of General Education, Univ. of Tokyo, Tokyo)
5. On the vasodilator action of efferent "sympathicus via posterior root"—sympathetic double innervation of bullfrog's blood vessels (II). K. Kotsuka (Dept. of Physiol., Kansai Med. Coll., Osaka)
6. Chronic thoracic duct-venous shunt preparation in dogs. F. R. Steggerda (Dept. of Physiol, Illinois Univ., U. S. A.)

Lecture by the President

On the vaso-motor nerves. Y. Nishimaru (ABCC), H. Hatta, and Y. Kishi (Institute of Angiology, Hiroshima)

Lecture by the Chairman

Studies on the intravascular blood coagulation. Y. Hashimoto (1st Surg. Dept., Univ. of Nagoya, Sch. of Med., Nagoya)

NOTICE FROM

THE JAPANESE HEART JOURNAL ASSOCIATION

The Second Department of Internal Medicine
Faculty of Medicine, University of Tokyo
1 Motofuji-cho, Bunkyo-ku, Tokyo

The Japanese Heart Journal has been published quarterly since January, 1960. From 1962, it will be published bimonthly and the annual subscription rate will rise to U.S. $12.00.

The Japanese Heart Journal Association will be happy to receive any advice or criticism from the readers concerning the contents of the journal.